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MICROSCOPIC CHANGES OF CERTAIN ANEMIAS DUE TO RADIOACTIVITY*

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In 1925, we described the etiology, symptomatology, pathology and physicochemistry of a series of occupational poisonings due to the ingestion of radioactive substances, especially aged mesothorium, which occurred in the watch dial industry.[1] These cases occurred among girls employed in painting the dials of watches and clocks with luminous paint, the paint entering the body by way of the gastrointestinal tract as a result of the habit of pointing in their mouths the tips of the brushes used in painting.

Many of the patients observed showed a leukopenic anemia of the regenerative type, with, or without, necrosis of the jaw developing sometimes years after they had stopped their work as dial painters.

In a fatal case we demonstrated, by means of the electrometer, gamma radiation from the body during life and measurable amounts of emanation in the expired air. In the organs after death, amounts of radioactive elements were found, sufficient to be determined quantitatively by alpha radiation and penetrative gamma rays, notably in the bones, spleen and liver.

This report was published as a warning that when long lived radioactive substances are introduced into the body by way of the gastrointestinal tract, death may follow a long time after, from the effects of constant irradiation on the blood-forming centers. Minute particles of the radioactive substances undergo phagocytosis by the local and migratory histiocytes of the reticulo-endothelial system and are deposited in the storage organs in sufficient amounts to produce, for a period of

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1. Martland, Harrison S.: Some Unrecognized Dangers in the Use and Handling of Radioactive Substances (with special reference to the storage of insoluble products of radium, mesothorium, etc., in the reticulo-endothelial system), New York Path. Soc., Oct. 8, 1925. Martland, Harrison S.; Conlon, Philip; and Knef, Joseph P.: Some Unrecognized Dangers in the Use and Handling of Radioactive Substances, J. A. M. A. **85**:1769 (Dec. 5) 1925.

time, seemingly curative or stimulative reaction, to be followed later by exhaustion and destruction of the blood-forming center.

Since this first report, we have had further opportunity to study a case which at that time was reported as a chronic leukopenic anemia of the pernicious type with necrosis of the jaw. The patient's expiratory air showed a measurable amount of radioactivity. She finally died, and the histologic and chemicophysical examinations confirm and add additional support to our opinions expressed in the original report. In addition we have had time to make a more detailed examination of the tissues, especially as regards the type of anemia and the distribution in the body of the radioactive substances.

The patient, aged 24, had worked for seven years as a dial painter, during which time she pointed brushes with her lips. She developed a chronic anemia with extensive necrosis of the inferior maxilla. Her blood pictures were characterized by a profound anemia, in which the color index was usually one plus, by a large cell anisocytosis with many macrocytes, by slight polychromatophilia and occasional granular basophilia, and by the occurrence of a scant number of nucle-

Fig. 1.—Femurs showing marrow cavities. Normal femur and femur showing marked erythroblastic regeneration. The replacement of the adult fatty marrow by deep red marrow may be noted.

ated red cells and a rare megaloblast. Blood platelets were abundant in smears; there was no purpura or bleeding. The icterus index was normal and the van den Bergh tests were negative, showing that while the anemia resembled closely that of addisonian anemia, there was little or no evidence of hemolysis. She died after an illness of about three years from a progressive anemia with extensive necrosis of the lower and upper maxillae, and a terminal bronchopneumonia.

Necropsy showed an intense replacement of the normal adult fatty marrow by a red regenerating bone marrow (fig. 1). The marrow of the femurs was dark red throughout, and the lesions more pronounced than that seen in the most characteristic case of pernicious anemia. Histologically the marrow was characterized by an enormous number of nucleated red cells, normoblasts and megaloblasts, which showed a regeneration of the megaloblastic type (fig. 2). The megakarocytes were normal, and the eosinophils were present in great numbers. The marrow was characterized also by the small amount of hemosiderin deposits. In the spleen, liver, heart and kidneys, the hemosiderin deposits were either absent entirely or present only in very small amounts. This is contrary to the observations in most cases of addisonian anemia.

The organs after incineration were tested for radioactivity, and the results are summarized in table 1.

It will at once be seen that the main storage of radioactivity was in the bones. The spleen and liver, also important storage organs of the reticulo-endothelial system, contained radioactive substances, but in very small amounts. The rest of the organs contained practically no

TABLE 1.—*Determination of Radioactivity in Incinerated Organs with the Alpha Electric Scope*

Organ	Incinerated	
Bones	3 days after	220 subdivisions per hour more than normal leak
	1 month after	365 subdivisions per hour more than normal leak
Spleen	1 month after	40 subdivisions per hour more than normal leak
Liver	1 month after	30 subdivisions per hour more than normal leak
Gastro-intestinal tract	1 month after	23 subdivisions per hour more than normal leak
Other organs	1 month after	Practically no radioactivity

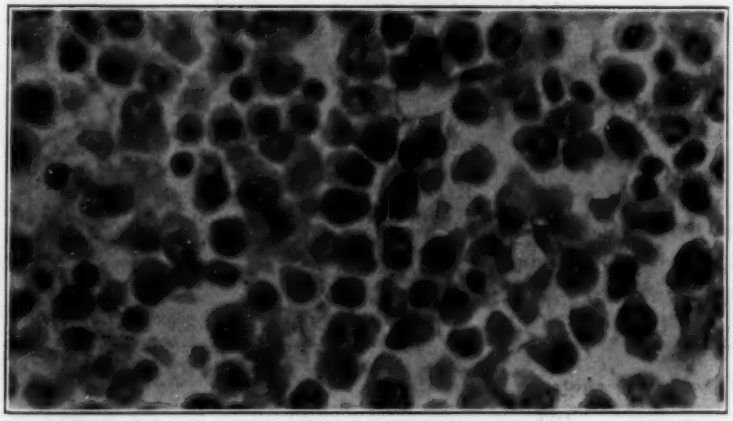

Fig. 2.—Histology of marrow. A regeneration of the megaloblastic type may be noted.

radioactive substances. The gastro-intestinal tract, however, which was the portal of entry, still showed an almost negligible amount. When it is recalled that these cases were observed long after the ingestion of the radioactive substances, it is apparent that we are dealing now with the effects of permanent storage. Long ago these substances had disappeared from the other organs of the body. The main storage of the radioactive substances was in the reticulo-endothelial system of the bone marrow sinusoids, and finally the bone cortex. Its close proximity to the erythroblastic and leukoblastic centers, the actual distance often being a matter of microns, exposed the blood-forming centers to a constant bombardment of not only beta and gamma rays, but also the short penetrating alpha rays which form over 80 per cent of the total radium

energy, and which are a great deal more destructive in their action on the tissues. This constant irradiation may go on for years before the centers are finally exhausted. The amount needed to exhaust them may be very small. Therefore, it is impossible to state what a lethal dose in this disease may be. This, it will be observed, is contrary to most poisonings in which the lethal dose is always a point of contention among the toxicologists.

Since the bones contained the largest amount of radioactivity, a chemical separation was then undertaken, with the results summarized in table 2.

The very small amount of radioactive substances required to produce a fatal outcome may be noted, and also the fact that mesothorium plays the more important rôle. Mesothorium, in equilibrium with radiothorium, emits 5 alpha particles, whereas radium emits only 4. The alpha particles of mesothorium, and its decayed products, have a greater

TABLE 2.—*Chemical Extraction*

1. Bones.....	Incinerated in electric oven to white ash with excess of carbon	
2. Ash.....	Paste made with barium chloride and hydrochloric acid. Boiled with hydrochloric acid and distilled water	
3. Filter.....	Filtered while hot through hard paper	
	Precipitate	Filtrate
	(a) Paper dried and incinerated (b) Radiothorium (c) Radioactivity measured 30 days later (d) Tubed	(a) Sulphuric acid added (b) Let stand for precipitate (c) Boil and filter while hot to hold calcium sulphate in solution
4. Second filtering		
	(a) Paper dried and incinerated (b) Radium and mesothorium (c) Radioactivity measured 30 days later (d) Tubed	(a) No radioactivity if extraction is complete

Result: About 150 micrograms were found in the entire skeleton, 70 per cent being mesothorium

velocity and penetration than those of radium, and, therefore, are chemophysically and physiologically more active.

A further test of the presence of radioactive substances in the bones was made by strapping dental films, enclosed in their original lightproof packets to the bones and interposing between the surface of the bone and the film, metallic clips and other objects. After exposure the films were developed. Distinct shadowgrams of the metal clips were obtained from the femur after seven days' exposure, and from the inferior maxilla in eighteen days. Following the appearance of our former papers on the subject, which were abstracted in French by Lacassagne,[2] we received from him a communication describing the results of his experiments with the distribution of polonium after intravenous injections. Lacassagne and his co-workers in the Radium Institute of

2. Lacassagne, A.: Un nouvel accident professionnel des manipulateurs de corps radioactifs: la necrose des maxillaires, Paris méd., Feb. 6, 1926, no. 6, pp. 132-135.

the University of Paris injected polonium into dogs intravenously. Polonium is radium F, a decayed product of radium emitting only alpha rays. About ten days after injection the animals died or were killed. The paraffin blocks, from which histologic sections of the various organs were made, were placed against photographic plates after the required number of sections were cut. In from fifteen to twenty days, "Autohistoradiographies" were obtained from the organs containing the polonium, and the exact location of the particles in the tissues could be demonstrated and compared to the corresponding histologic section.[3]

This ingenious technic enables us to locate very small amounts of radioactive substances in the tissues, which could not be demonstrated in any other way. It must be remembered that large amounts of polonium were injected into his dogs by way of the veins, and that after ten days, when the dogs died or were killed, the polonium would be present in considerable amounts, not only in the organs of the reticuloendothelial system but practically in every organ of the body, and espe-

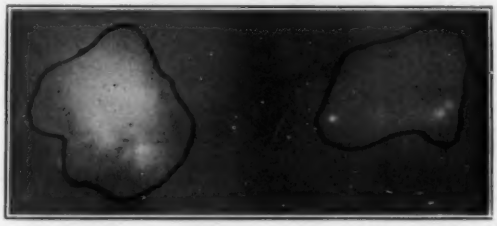

Fig. 3.—Autohistoradiography from inferior maxilla. Obtained from bone in twenty-one days' exposure. The irregularity of distribution in the two pieces of bone may be noted.

cially the organs of elimination. The amount and distribution of radioactive substances in our cases, therefore, cannot be compared to the conditions under which Lacassagne performed his experiments. We do not expect to get such large amounts and such wide distribution. Lacassagne's methods, however, were tried with the following results: Paraffin blocks of heart, liver, spleen, kidneys and gastro-intestinal tract after from four to five weeks' exposure against photographic plates showed no radioactivity. Paraffin blocks of femur and inferior maxilla gave definite exposures in twenty-one days.

Two interesting conditions are worthy of note. First: The very unequal distribution of the radioactive substances in the bones; second, the location of the deposits. If we examine the autohistoradiography from the inferior maxilla, we shall note (fig. 3) that instead of an even

3. Lacassagne, A.; Lattes, Mme. J., and Ladivan, J.: Etude expérimentale des effets biologiques du polonium introduit dans l'organisme, J. de radiol. **9,** no. 1, January, 1925.

distribution, the radioactive substances are deposited in scattered concentration points, and not stored diffusely throughout the bone. These points are so concentrated in places that we have tried to check up the histologic observations around them. In the deposit of any metallic poison in the bones from the circulation we would naturally expect that the metals would undergo phagocytosis by the histiocytes of the bone marrow, and from there would be deposited into the dense cortex of the bone by some route about which we are in ignorance. This is the first time, we believe, that this irregular deposit has been proved, and it brings up an important question in toxicologic examinations.

The same manner of storage should be expected in chronic poisoning with other metallic elements, for instance arsenic trioxide. The common technical procedure in the toxicologic examination of the bones in a homicide case for arsenic is to estimate the amount of arsenic in, say

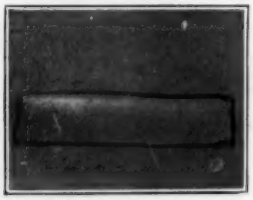

Fig. 4.—Autohistoradiography from femur. Obtained from bone in twenty-one days' exposure. The largest storage in the cortex near the periphery of the bone may be noted.

200 Gm. of bone, and then to estimate roughly the weight of the skeleton from the height and weight of the body. The amount of arsenic found in 200 Gm. of bone gives a basis for estimating the total amount in the entire skeleton. If arsenic trioxide is stored in the same manner as these radioactive substances, and we have every reason to believe that it is, the technical fallacy of this method is obvious.

If we examine the autohistoradiography of the femur (fig. 4), we shall see that there is a tendency for the largest part of the radioactive substances to be stored in the dense cortex near the periphery of the bone, instead of toward the marrow cavity. Why this is so we do not know, and it opens up an important channel of investigation into the actual anatomy and function of the cortex of the bone.[4]

4. These investigations have been recognized in American and foreign literature, especially the French. As a result, the liability has been recognized and the Compensation Laws of the State of New Jersey amended so as to protect in the future and give adequate compensation to workers in radioactive substances who develop occupational poisoning.

SUMMARY

Suspicion was first called to occupational radium (mesothorium) poisoning in the watch-dial industry by Blum [5] in 1924, who reported before a dental society a case showing an unusual and intractable osteomyelitis of the mandible and maxilla in a worker using luminous paint. Hoffman,[6] in May, 1925, reported from the point of view of a statistician five death and twelve living cases, occurring among dial painters who had developed resistant infections of the jaw with buccal lesions and marked anemia. He was of the opinion that they represented an unusual form of occupational poisoning. Castle and the Drinkers,[7] in August, 1925, reported five cases of necrosis of the jaw among employees engaged in the application of luminous paint which had been included in Hoffman's paper. After an investigation into the manufacture of this paint, an inspection of the factory and employees, they concluded that there was undoubtedly proof of excessive exposure to radiation in these workers, especially from inhalation of particulate material containing a radium salt. They did not ascertain the presence of mesothorium in this paint. From an industrial point of view, they concluded that the subject was not finally proved but that the safest course would be to assume that an occupational poisoning existed, and they made recommendations for the future protection of workers.

Our original paper,[1] therefore, actually described for the first time the etiology, symptomatology and pathology of these cases. The present report adds additional confirmation of the views expressed in that paper. Radioactivity was demonstrated in the expired air by means of electrometers. The only previous demonstration of emanation in the exhaled air in the human being that we know of was made in 1915 by Seil, Viol and Gordon [8] after the experimental ingestion of 50 micrograms of radium (element) by mouth. They stated: "Radium taken into the body is continually disintegrating with the formation of the gaseous radioactive substance, radium emanation. This gas is carried by the blood to the lungs, where the emanation passes out of the blood by diffusion, and is lost from the body in breathing." Their experimental work adds further strength to our conclusions. In our cases

5. Blum and Theodore: Osteomyelitis of the Mandible and Maxilla, Am. Dental Ass., September, 1924.

6. Hoffman, Frederick L.: Radium (Mesothorium) Necrosis, J. A. M. A. **85**:961 (Sept. 26) 1925.

7. Castle, W. B.; Drinker, Katherine R., and Drinker, Cecil K.: Necrosis of the Jaw in Workers Employed in Applying a Luminous Paint Containing Radium, J. Indust. Hyg. **7**:371 (August) 1925.

8. Seil, Harry A.; Viol, Charles H., and Gordon, M. A.: The Elimination of Soluble Radium Salts Taken Intravenously and Per Os, Radium **5**:42 (May) 1915.

radioactive substances were demonstrated qualitatively and quantitatively by means of electroscopes and electrometers in the various organs of the body after death before and after chemical extraction, especially in the main storage organs of the reticulo-endothelial system, namely: the bones, spleen and liver. Again the only previous measurement of radioactivity in human organs was made in December, 1913, by Cameron and Viol [9] in a patient dying of cancer three and one-half months after the experimental intravenous injection of 1 mg. of radium element. The largest amounts were found in the bone marrow, liver and lungs. In addition, the presence of radioactivity was further demonstrated in our cases by photographic methods. Shadowgrams of metal clips, etc., were obtained from the bones on dental film by exposure to beta and gamma rays coming from the bones. Photographs were also obtained directly on photographic plates by direct contact with the bones from alpha, beta and gamma rays.

By means of an ingenious technic used by Lacassagne in his experimental work with polonium, we were able to obtain autohistoradiographies from paraffin blocks of the bones after histologic sections were cut. These demonstrated the uneven deposit of the radioactivity in the bone and a peculiar concentration in the femurs toward the outer part of the cortex. It is possible that in the storage of other poisonous elements the same unequal distribution occurs. If this is so, it is of great importance from a toxicologic standpoint, since it might very well occur in arsenical poison.

These studies throw considerable light on the type of anemias occurring among workers in radioactivity. Our cases have all been of the regenerative type from a morphologic standpoint resembling true pernicious anemia, but with the difference that there is absence of evidence of hemolysis as shown by the absence of a hyperbilirubinemia and by very little hemosiderin deposits in the important organs. Heretofore in the report of anemias due to radioactivity, Mottram, Weil, Lacassagne, Brule, et al., have reported these cases as anemias of the aplastic type showing little or no evidence of regeneration. As in practically none of these cases necropsy was performed and no study of the bone marrow was made, this conception is purely a clinical one, and in our opinion, is not substantiated by the facts as we know them.

These studies further emphasize the importance of the reticulo-endothelial system as a storage system for foreign particles of colloidal or near colloidal size. The independence of the reticulo-endothelial system from the adjacent hemopoietic system is also emphasized.

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9. Cameron, W. H., and Viol, Charles H.: Classification of the Various Methods Employed in the Internal Administration of Radium Emanation and Radium Salts, Radium **4**:66 (Jan.) 1915.

SYPHILIS OF THE LUNG

WITH A REPORT OF A CASE *

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CHICAGO

"Phthisis a lue venera," was a fairly common diagnosis in the sixteenth, seventeenth and eighteenth centuries, and Paré, Borehare, Laennec, Morgagni, DePaul and Virchow are some of the men who recognized the condition. Following the clear differentiation of syphilis from gonorrhea and soft chancre by Ricord,[1] and the discovery of the tubercle bacillus by Koch in 1882, skepticism marked the attitude of most clinicians and pathologists toward pulmonary lesions previously considered syphilitic. The widespread application of the Wassermann test, and the discovery of *Spirochaeta pallida* have greatly increased the reliability of diagnoses in the twentieth century. Warthin's [2] high figures for incidence of syphilitic infection suggest the possibility of frequent pulmonary involvement.

A review of the literature on pulmonary syphilis reveals about five generally accepted types of the disease in the acquired form: the gumma, according to Warthin, the rarest lesion, but still the one on which the frequency of syphilitic infection in an organ is based; chronic interstitial pneumonia, gradually leading to another type, extensive pulmonary sclerosis; bronchopneumonia, and a mixed form sometimes called "syphilitic phthisis," in which pneumonia, gummas, fibrous changes, cavity formation and bronchiectasis may coexist with gangrene at times added where there is secondary infection.

Elizalde,[3] in an extensive study of pulmonary syphilis in thirty adults, describes four stages which he thinks are well defined and analogous to the stages of lobar pneumonia but slower in evolution. The first stage is characterized by an acute productive process, vascular dilatation, exudation, predominantly serous, with a little fibrin perhaps, and a few leukocytes, so there is a beginning consolidation. A little later there is a destruction of the leukocytes by clasmatocytes, and then a multiplication of fibroblasts in the interalveolar spaces and about the blood vessels and bronchi. Small lymphocytes are little in evidence and plasma cells are rare. The exudation after a time reduces the caliber of the

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1. Ricord, P.: A Practical Treatise on Venereal Diseases, trans. by S. Doane, 1852, ed. 6, V. S. Redfield, Clinton Hall, N. Y.

2. Warthin, A. S.: The New Pathology of Syphilis, Am. J. Syph. **2**:425, 1918.

3. Elizalde, P. J.: Anatomia Patologica y Patogenia de la Sifilis Pulmonar, An. de Fac. de med., Montevideo **5**:315, 1920.

vessels, and then the second stage is at hand. A rapid change occurs in cellular types. Lymphoid cells of various sizes and plasma cells form special aggregations in the vicinity of the blood vessels and bronchi and have the microscopic characteristics of miliary gummas. The striking feature is that there is no new formation of blood vessels to nourish the cells which then decay. In the succeeding period there is a fragmentation of the elastic fibers of the blood vessels and bronchi and later a destruction so that the bronchi become dilated. Cellular regression and the reestablishment of alveolar permeability are the outstanding features of this stage. In the final stage the fibroblasts are small and have many fibrils. The plasma cells are also small. There are many kinds of lymphoid cells and many are in collections especially about the blood vessels and bronchi. Resolution begins peripherally. There is a desquamation of alveolar epithelium, and pieces of this may be expectorated. Foci of peribronchitis are common, and the lung is contracted.

In twenty-four of this author's cases the syphilitic process was unilateral, both lungs being attacked with similar frequency. Aortic aneurysm was present in 75 per cent of the bodies, causing bronchial compression, and the syphilitic lesion always occurred on the side compressed. Elizalde explains this localization on a basis of decreased pulmonary activity and resistance. In one instance trauma to the lung parenchyma by streptococci with a subsequent anaerobic growth of spirochetes seemed the most plausible explanation. Gummas were invariably found in relation to a blood vessel, and even when numerous spirochetes were found in the interstitial tissue only a pneumonia resulted in the absence of a vessel.

Gross descriptions of the lungs in pulmonary syphilis are available, and the following is that of Germain Sée [4]:

> The lungs are as it were ploughed with deep furrows, filled with sclerous tissue. These furrows resembling syphilitic cicatrices of the liver, deform the surface of the lung determining more or less marked retraction of the parenchyma. . . . Nodosities are perceptible . . . appearing at the surface or disseminated in the depths of the parenchyma. . . . The alterations are the less pronounced the more the nodosities are wanting and all may be limited to a circumscribed thickening of the pleura and to fibrous bands partitioning off a portion of the lung.

Fowler [5] cites the following gross differences between syphilis and tuberculosis of the lung:

> Tubercle usually affects the apex of the lung and subsequently the apex of the lower lobe and tends to progress in a certain route. The primary lesion of

4. Sée, G.: Diseases of the Lungs, New York, William Wood and Company, 1885, pp. 260-267.

5. Fowler, in Osler and McCrae: Modern Medicine, ed. 2, Philadelphia, Lee and Febiger, 1914, pp. 171-174.

syphilis is often about the root and central part of the lung. The disease follows no definite line of march and gummata may be found in any position. Both tuberculosis and gumma may undergo either necrosis and caseation or fibrous transformation, but with caseous tubercle the tendency toward softening and cavity formation is the rule, whereas a caseous gumma very rarely breaks down. The progressive destruction of the lung, by a process of disintegration leading to a gradual increase in the size of a cavity, a change so commonly observed in tuberculous disease, is rarely observed in syphilis except as a secondary result of stenosis of one of the main bronchi. In nearly all cases of advanced destruction of the lung, occurring in the subjects of syphilis, stenosis either of the trachea or of one of the main bronchi is present, whereas this lesion is very rare in tuberculosis. The cavities found in cases of pulmonary syphilis are usually bronchiectatic, but not invariably so; whereas in tuberculosis they are commonly due to progressive destruction of the lung but may be bronchiectatic. The tendency to the formation of pulmonary aneurysm, so marked a feature in tuberculosis is rarely observed in pulmonary syphilis.

Carrera [6] describes the scar of tuberculosis as:

. . . round, sharply delimited with concentric fibers, hyaline and scant in nuclei, devoid of vessels and elastic tissue, less given to anthracotic pigmentation but more frequently calcified and very often confluent. The syphilitic scar is irregular, radiating, more like ordinary connective tissue, still contains blood vessels often with angiectatic capillaries, continuous with the thickened walls of the nearest alveoli, still showing elastic fibers and the outlines of old vessels and alveolar walls and extremely rarely conglomerate. The most conclusive differential point is the finding of collections of plasma cells in the fibrosis of syphilis.

The histologic differences between tuberculous and syphilitic lesions of the blood vessels have been described in detail by Carrera, and a brief statement of the essential features of each follows. In tuberculosis there is a rather generalized destructive process; the elastic tissue degenerates and does not proliferate in the intima and the muscle fibers of the media degenerate or atrophy. In syphilis the elastic tissue is better preserved and at times is markedly increased in the intima; "round cell" infiltration in the intima is more marked than in tuberculosis and is seen as a distinguishing feature in the media, and in the adventitia there is plasma cell accumulation and new capillary and new "connective tissue" formation.

Warthin,[2] in reporting his "New Pathology of Syphilis," determined syphilis of the lung positively in three cases, but admitted he had not devoted as much time to this organ as he thought necessary. He maintains that the difference in incidence of syphilis recorded is mainly due to a difference in pathologic criteria, and statistics appear to prove this. Claytor,[7] in 1905, found no pulmonary syphilis in 13,000 specimens at

6. Carrera, J. L.: Syphilis of the Lungs, Pathological Study of the Lungs in 152 Autopsy Cases, Am. J. Syph. **4**:1 (Jan.) 1920.

7. Claytor, T. A.: Syphilis of the Lung, with a Report of the Anatomical Findings in a Case, Am. J. M. Sc. **129**:563, 1905.

the Army Medical Museum in Washington. Symmers,[8] in 4,880 necropsies at Bellevue Hospital, New York, reported syphilis of the lung in 0.24 per cent. Carrera,[6] working in Warthin's laboratory, found microscopic evidence of pulmonary syphilis in 7.8 per cent of 152 bodies having syphilis elsewhere. Osler [9] begins a paragraph on syphilis of the lung with "This is a rare disease," and continues: "In 2,800 postmortems at the Johns Hopkins Hospital there were twelve cases with syphilitic disease in the lungs; in eight of these the lesions were in congenital syphilis." This is an incidence of 0.42 per cent for the total number of cases.

Lord [10] saw only one instance of "indurative pneumonia" associated with syphilitic ulceration of the trachea in 3,000 necropsies at the Massachusetts General Hospital and was doubtful as to the syphilitic character of the lung lesion. Howard,[11] at the University of Iowa hospital, found that 0.55 per cent of 720 bodies on which necropsy was performed had pulmonary syphilis clearly demonstrable histologically, although *Spirochaeta pallida* was not found. This author states that the incidence of pulmonary syphilis as compared with visceral syphilis elsewhere and exclusive of the nervous system is 1 : 20.

Clinical observers generally record a higher incidence of pulmonary syphilis than pathologists, with the exception of Carrera. Such conclusions are based on an absence of tubercle bacilli in the sputum, positive Wassermann reactions, histories of infection coupled with marked improvement under antisyphilitic treatment and roentgen-ray shadows presumably characteristic in location and configuration. Watkins [12] claims the following roentgenologic differences between syphilis and tuberculosis of the lung:

> Tuberculosis tends to invade the upper lobes. The characteristic shadows are found surrounding the apical or subpleural lobules. The shadows . . . show a definite relation to some branch of the bronchial tree. Syphilis tends to invade the lower and middle lobes and to involve the tissue at the hilum first so that the densest shadow is there, diminishing toward the periphery. The shadows do not bear a distinct relation to the bronchi and at their edges have either a ragged, worm eaten appearance or the lung is evenly speckled; or there are lance-like radiations of dense elastic tissue.

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8. Symmers, D.: Late Acquired Syphilis, J. A. M. A. **66**:19 (May 6) 1916.

9. Osler, Sir William, and McCrae, T.: The Principles and Practice of Medicine, ed. 9, 1920, New York, D. Appleton & Company.

10. Lord, F. T.: Diseases of the Bronchi, Lungs and Pleura, Philadelphia, Lea and Febiger, 1915.

11. Howard, C. P.: Pulmonary Syphilis, Am. J. Syph. **8**:1, 1924.

12. Watkins, W. W.: Roentgen Diagnosis of Lung Syphilis, Am. J. Syph. **1**:760, 1917.

This author rationalizes his interpretations in the light of Carrera's work subsequently.[13]

The Karschners,[14] in 120 cases selected from the literature, in fifty-five of which postmortem examinations were made, found pulmonary syphilis in the ratio of males to females 2:1 and the average time of appearance eleven years after the initial lesion. Munro[15] found "The shortest time between infection by the spirochete and symptoms referable to the chest 3.5 years." He bases this statement on a 6 per cent incidence of pulmonary syphilis found in 100 patients examined as a routine at Glenlomond, a tuberculosis hospital. Stanley,[16] basing his conclusions on somewhat more representative figures, says:

> In a series of 1,000 cases, notified as phthisis, I found two certain of syphilis and one doubtful. In the last seven years I have collected fifteen cases, some of which have come to postmortem examination and some are still under observation—observation which confirms the opinion that they are syphilis and not tuberculosis.

The general attitude toward syphilis of the lungs is apparent from the following statement of Blakeman[17]: "In the presence of syphilitic stigmata, any obscure lung pathology should be considered as syphilitic until proven otherwise." Reports of cases not confirmed by necropsies are naturally of far less significance than those tested out by rigorous histologic methods. The impossibility of applying such methods to patients is apparent, but a greater reticence to accept a case as syphilitic without long, careful observation will lead to a more accurate, reliable compilation of data on an interesting disease process.

REPORT OF CASE

History.—E. L., aged 42, occupation unknown, while riding with a friend in an automobile and apparently in good health, suddenly became ill and died on the way to the Cook County Hospital.

Anatomic Diagnosis (Dr. E. R. LeCount).—Marked syphilitic aortitis; fusiform aneurysm of the root of the aorta; "cor bovinum," giant papillary muscles; huge syphilitic scars of the lungs with compensatory bullous emphysema; syphilitic hepatitis with scarring; scar of the penis; dilated mitral ring; moderate edema of the brain substance, lungs and tracheobronchial lymph glands; marked passive hyperemia of the abdominal viscera; engorged veins of the head; acute slight ascites and moderate bilateral hydrothorax; subpleural and subpericardial petechial hemorrhages; localized subperichondrial hemorrhages of the costal cartilages (agonal?); abrasions of the scalp and left leg; bruise of the right arm;

13. Watkins, W. W.: X-Ray Characteristics of Lung Syphilis, J. Radiol. **1**:297, 1920.

14. Karschner, R. G., and Karschner, C. F.: Syphilis of the Lungs, an Analysis of 120 Selected Cases, Ann. Med. **1**:371, 1920.

15. Munro, W. T.: Syphilis of the Lung, Lancet **2**:1376, 1922.

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feces-stained perineum; sclerosis of the aortic leaflets; protruding hemorrhoids; marked bilateral fibrous orchitis; chronic catarrhal prostatitis; cysts of the lining of the prostatic part of the urethra; fibrous adhesions between (1) the visceral and parietal layers of the tunica vaginalis sacs, (2) the spleen and diaphragm, (3) the gastrohepatic ligament and gallbladder, (4) the aorta and pulmonary artery; localized bilateral fibrous pleuritis; epitheliosis of the esophagus; hyperplasia of the spleen, inguinal and tracheobronchial lymph glands, lymphoid tissue of the back of the tongue and circumvallate papillae; slightly flaring costal margins; fenestrated pulmonary valve leaflets; vertical furrows of the right lobe of the liver; retrocecal appendix vermiformis, directed upward; moderate hypostatic hyperemia of the lining of the bowel.

Necropsy.—The aorta was thin and dilated at its root and arch so that as it lay in the pericardial sac its wall was folded longitudinally in two places. The circumference of the aorta was 13 cm., 2 cm. distal to the free margins of the aortic leaflets; 7.3 cm. at the distal margin of the innominate artery; 5.4 cm., 2 cm. proximal to the celiac axis and 3.8 cm., 2 cm. distal to the renal arteries. The adventitia of the root of the aorta was pink from a delicate tracery of engorged blood vessels and for its first 6 cm. was stippled with petechial hemorrhages (about 15 per square centimeter). The sinuses of Valsalva were dilated so that there were vertical furrows in the outer surface of the aorta between the pouches opposite the mutual attachments of the aortic valve leaflets. The lining of the sinuses for the coronary arteries was finely wrinkled radially from the mouths of these vessels. The lining of the arch of the aorta was coarsely wrinkled longitudinally and mottled about 20 per cent generally by calcified plaques up to 15 by 10 mm. The linear wrinkles became gradually finer distal to the arch and ended abruptly 10 cm. up from the superior mesenteric artery. The abdominal part of the aorta was coarsely wrinkled for 4 cm. distal to the superior mesenteric artery and between the renal arteries.

The heart with 1.2 cm. of attached aorta and the pulmonary artery to its bifurcation weighed 562 Gm. The subepicardial fat was most abundant in the front of the right ventricle and close to the septum; it was 18 mm. thick at the coronary sulcus. The myocardium was light red-brown and grossly free from fibrous change. There was nothing but fluid blood in the right ventricle. The moderator band was well developed and about 9 mm. in diameter. There were about five fenestrations of each pulmonary valve leaflet near their contiguous edges, the largest 8 by 5 mm. The adventitia and intima of the pulmonary artery were finely wrinkled longitudinally and the lining, in addition, was almost imperceptibly pitted. The left ventricle was firm, with rigor. The mitral ring admitted five fingertips easily. The circumference of the mitral ring was 11.6 cm.; of the tricuspid, 14.5. The maximum thickness of the outer wall of the left ventricle was 31 mm.; of the right, 7. The papillary muscles of the left ventricle were 2.5 by 1.8 cm. at their bases. The free margins of the aortic valve leaflets were from 2 to 3.5 mm. thick, gray-yellow and smooth. The anterior and right posterior aortic leaflets were fused for 12 mm. vertically, and there was a similar fusion of the right and left leaflets for 7 mm. The mitral leaflets were pink-yellow from slight fatty thickening for 8 mm. in diameter at their centers, and their free edges were normally smooth. The coronary arteries had patent mouths, wide lumens and linings thickened by faint linear ridges. There was a wide anastomosis between the circumflex branch of the posterior coronary artery and the anterior coronary artery.

The upper lobe of the right lung was puckered and firm in a rough S-shape of the dorsal part of its thoracic surface; each arm of the S was about 7 cm.

long, and it was about 5 cm. high (fig. 1 *A*). This scar was about 1 cm. deep, and coarse indentations of a similar depth or less connected the arms of this *S* and also radiated from them (fig. 1 *B*). The depressions were blue-black, the intervening knobs of tissue pink-gray and firm. The posterior surface of the upper lobe near its root was irregular with alternating purple depressions and pink elevations in which the air sacs were up to 2.5 mm. in diameter. The middle lobe of the right lung was the largest (fig. 1 *C*) and was crepitant throughout.

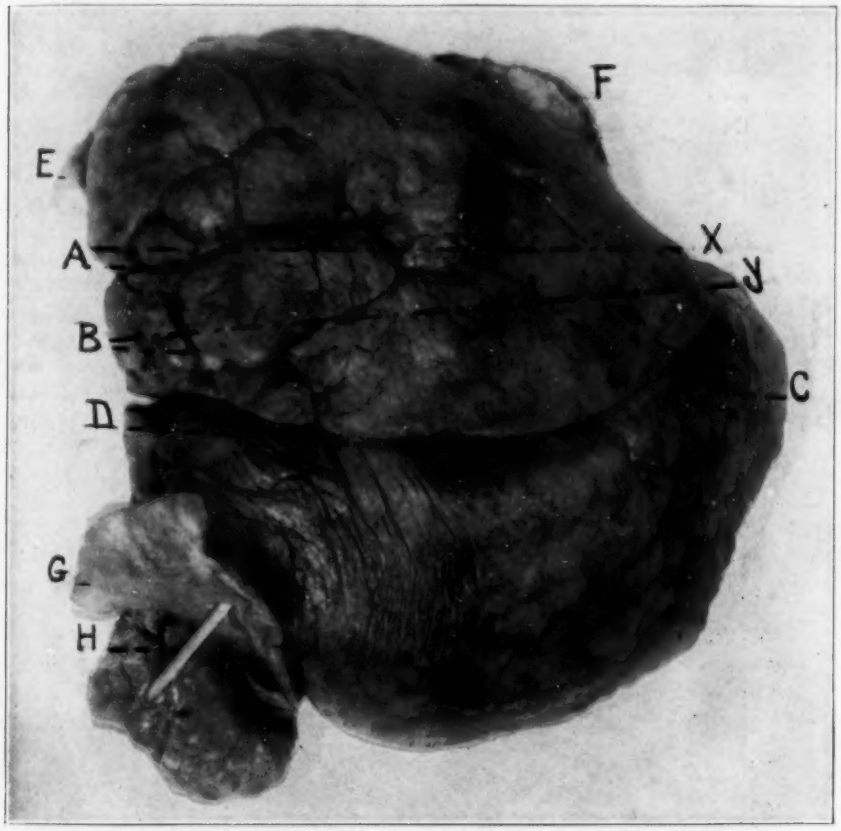

Fig. 1.—The costal surface of the right lung about two-fifths natural size showing: *A* and *B*, the scarring of the upper lobe; *C*, the large middle lobe; *D*, the adhesions between the upper and middle lobes; *E* and *F*, the adhesions at the apex and ventral edge respectively; *G*, the largest bullous component of the lower lobe; *H*, the scar tissue stalk of the bullous components; *X* and *Y*, the location of the photographs in figures 3 and 4, respectively.

There was a firm mass of adhesions about 2 cm. in diameter between the upper and middle lobes near the peripheral part of the dorsal third of the upper interlobar fissure (fig. 1 *D*). There were also dense patches of fibrous adhesions between the apex of the upper lobe (fig. 1 *E*) and between the ventral edge and mediastinum (fig. 1 *F*). The lower lobe was about one-eighth the size of the median and consisted of about five earlike parts, the largest 6 cm. in diameter

and 1 cm. thick (fig. 1 *G*), in which the air spaces were up to 3 mm. in diameter. These lobules were attached to a firm purple-black, shriveled stalk at the root of the lung (fig. 1 *H*). The right lung weighed 847 Gm.; the left, 392. Irregularly-shaped, smooth-topped projections up to 3 cm. in diameter (fig. 2 *A*) sharply defined by constricting bands of fibrous tissue formed the bulk of the

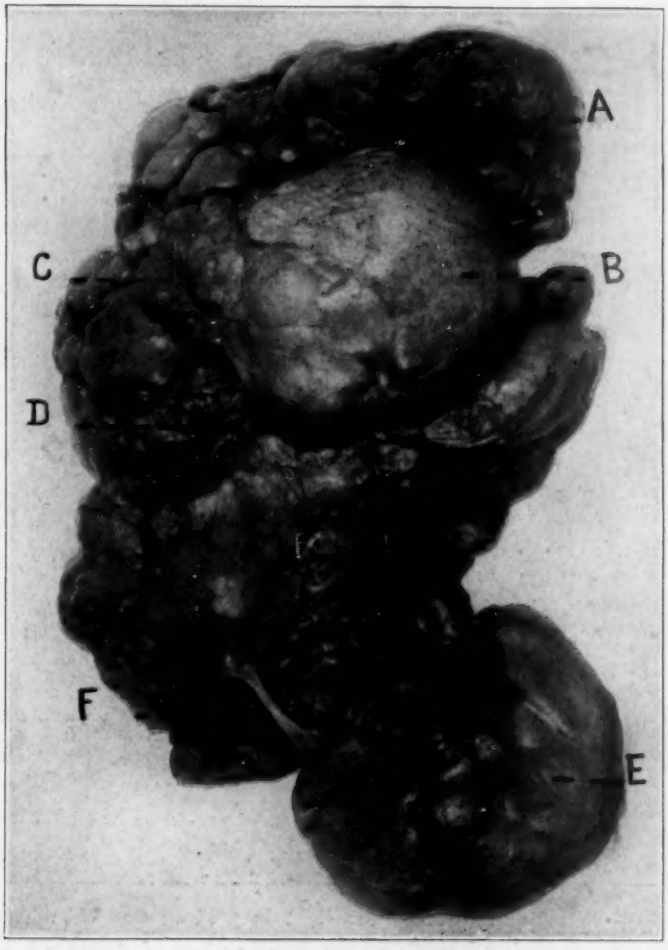

Fig. 2.—The costal surface of the left lung, about one-half natural size, showing: *A*, the irregular projections of emphysematous lung parenchyma; *B*, a huge bullous mass of the upper lobe; *C*, the pedicle of the mass; *D*, the position of the interlobar adhesions; *E*, a bullous mass of the lower lobe; *F*, a firm fibrous tissue strand between two giant emphysematous masses.

upper lobe of the left lung. The peripheral air sacs in these nodules were up to 3 mm. in diameter. Pendant from the center of the costal surface of this lobe was a feathery lavender-red mass 7 by 5.5 by 2 cm. (fig. 2 *B*), in which the air vesicles were up to 7 mm. in diameter. Its stalk was silver gray to black, 40 mm. wide and 2 thick (fig. 2 *C*). The lobes of the left lung were firmly bound together

by firm masses of fibrous tissue at the middle of the costal edge of the interlobar fissure (fig. 2 *D*). An emphysematous mass 8 by 6 by 2 cm. formed the lowermost part of the lower lobe (fig. 2 *E*) and was connected by a bridge of fibrous tissue 5 mm. wide and 30 long (fig. 2 *F*) to a similar bullous projection on the ventral edge of the lobe.

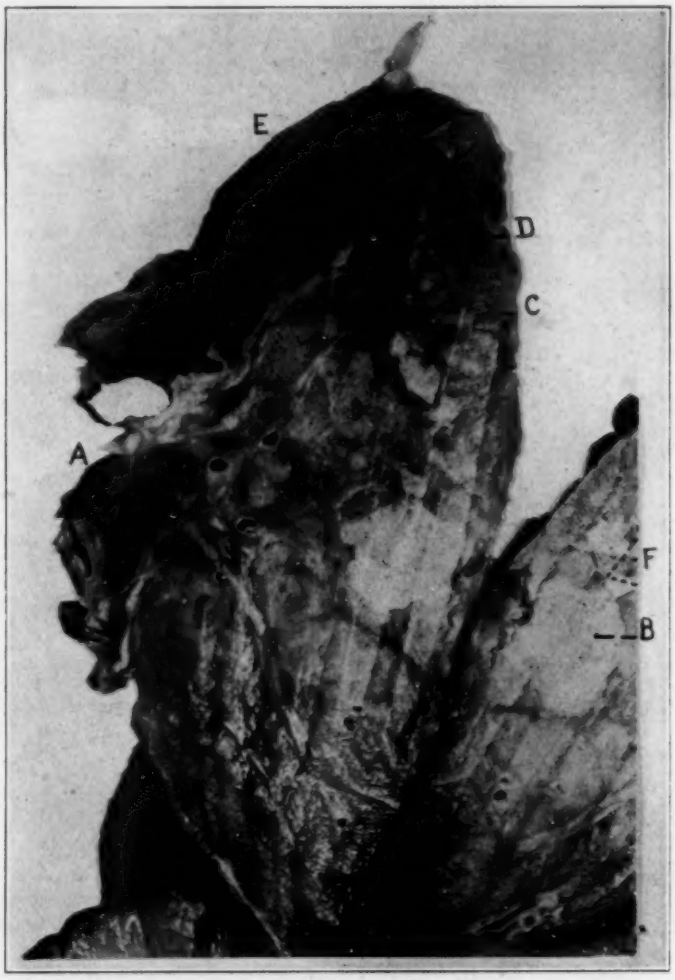

Fig. 3.—A surface formed by cutting the upper lobe of the right lung at "*X*," showing: *A*, the coal-pigmented nodule-containing lymph gland; *B*, the large nodules forming the larger part of this surface; *C*, the islands of dilated alveoli; *D*, the coal-pigmented mass; *E* and *F*, the location of microscopic sections.

The lungs were preserved in formalin after injecting formalin into the pulmonary veins with the arteries tied. On surfaces made by cutting the right lung at its apex there is a firm, slightly elastic yellow-gray nodule 22 by 15 mm. and one 7 by 5 mm. adjacent to it. The nodules are sharply defined, stippled

black, about one-fifteenth in the middle and bordered by firm, pink-gray fibrous tissue continued to puckered indentations of the lung periphery marked by coarse, radiating strands. There is a similar nodule although smaller (fig. 4 *A*) on a surface formed by cutting the upper lobe (fig. 1 *Y*). These pits (fig. 4 *B*) have

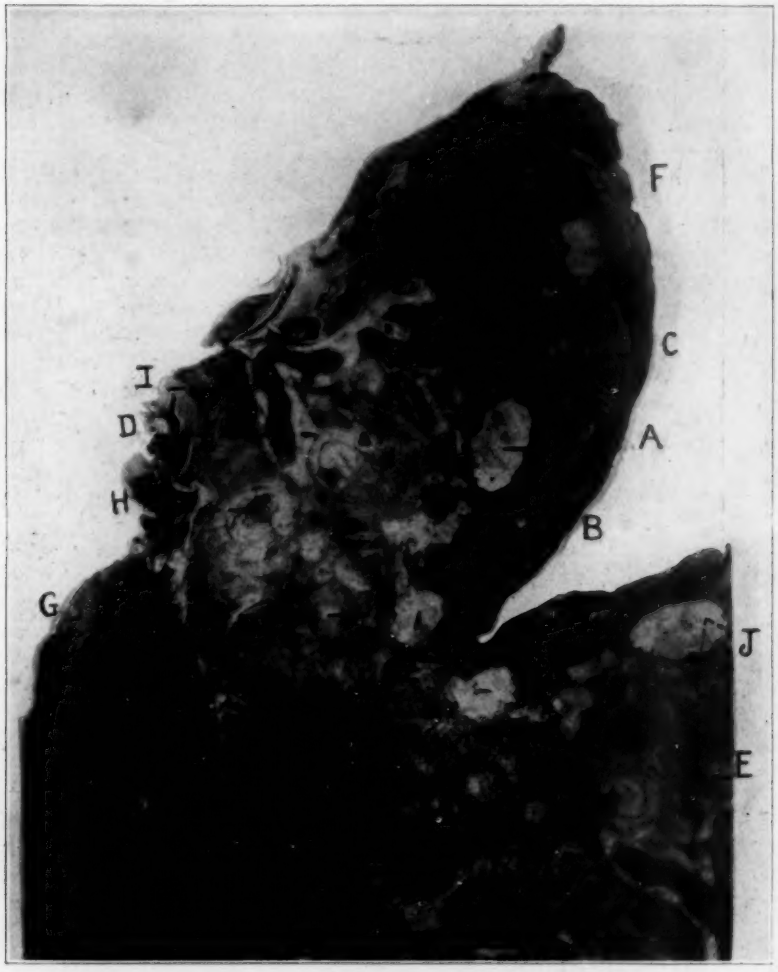

Fig. 4.—A surface formed by cutting the upper lobe of the right lung at *"Y,"* showing: *A*, a typical circumscribed and pigmented nodule; *B*, the puckered pleural pits; *C*, circumscribed emphysematous places; *D*, fibrous tissue masses about the bronchi, and *E*, those about blood vessels; *F*, compressed alveoli in the fibrous tissue; *G*, fibrous tissue ingrowth into a nodule; *H*, a friable and fissured nodule; *I*, markedly wrinkled bronchial mucosa; *J*, location of microscopic section.

markedly puckered walls and are up to 14 mm. deep. Between them are collections of air cells markedly dilated (fig. 4 *C*). Fibrous tissue strands up to 6 mm. wide, irregularly streaked and spotted with coal dust, border the bronchi (fig. 4 *D*) and bridge from these to encircle the blood vessels (fig. 4 *E*) and some of

the nodules described. In several places, as at *F*, figure 4, compressed and elliptical air sacs, their long axes parallel, are included in this fibrous tissue. A nodule 1 cm. in diameter near the hilum contains a branching ingrowth of fibrous tissue continuous with that about a neighboring artery and bronchus (fig. 4 *G*) and one near it (fig. 4 *H*), 12 by 9 mm., is yellow-brown, friable and fissured at its center. The walls of the blood vessels are markedly thickened, and some arteries with a wall 1 mm. thick have a lumen only 3 in diameter. The lining of the bronchi and bronchioles is markedly wrinkled transversely between longitudinal elevations of mucosa from 1 to 1.5 mm. apart (fig. 4 *I*). The peribron-

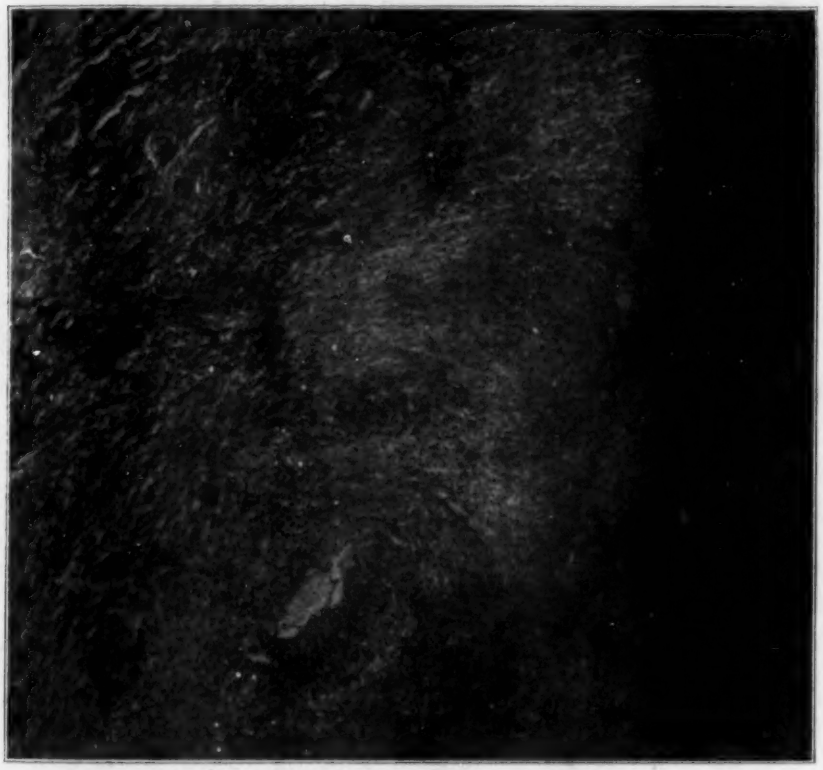

Fig. 5.—A low-power illustration of the edge of a gumma with small lymphocyte and plasma cell infiltration about a bronchiole.

chial lymph glands are about 90 per cent black from coal dust and very firm. One 6 by 12 mm. (fig. 3 *A*) contains two firm gray nodules each 1.5 mm. in diameter and uniformly stippled with coal dust. Surfaces formed by cutting the upper lobe at (*X*) about 1 cm. more cephalad than *Y*, figure 1, are about four-fifths firm nodules (fig. 3 *B*) and interlacing bands of fibrous tissue which hem in islands of air sacs dilated to 5 mm. in diameter and across which threadlike remnants of lung tissue branch (fig. 3 *C*). In one place near the dorsal border where three or four nodules coalesce there is a firm black place 1.5 cm. in diameter (fig. 3 *D*). The middle lobe of the right lung contains a black nodule 6 mm. in diameter on the middle of the front edge of its interlobar surface. There are

only one or two much pigmented nodules in the lower lobe. Because of its marked atrophy, there is an apparent increase in the arteries, which are laced together in many places by scar tissue. The air sacs of its bullous processes are uniformly 1.5 m.. in diameter.

Surfaces formed by cutting the left lung through both lobes vertically and close to the course of the main bronchi are mottled, about equally gray and pink and speckled with black. The upper lobe is about one-third interlacing bands of fibrous tissue up to 8 mm. wide and about one-third black from coal dust. About the lower and ventral one tenth of the upper lobe is fibrous tissue containing compressed air spaces, as mentioned in the right lung. Collections of these up to 14 by 11 mm. bounded by fibrous tissue form about one third of the surface, and 75 per cent of these sacs are 1 mm. in diameter. The upper two thirds of the front, 1 cm. of the upper lobe, contain air spaces up to 8 mm. in diameter. Nodules, 7 by 5 mm., partially encircled by coal deposits, gray except for slight black stippling and a little orange-yellow pigmentation at the center, are in the scar tissue 8 mm. from the lung apex and at the point of maximum puckering. The lower lobe is crepitant throughout, and its cells are barely visible grossly. Surfaces formed by cutting the earlike projections contain air sacs uniformly 3 mm. in diameter. The fibrous pedicles for these are remarkably twisted.

Paraffin sections stained with hematoxylin and eosin and phosphotungstic acid hematoxylin, in the upper lobe of the right lung show (fig. 4 *H*), in the fissured place a closely packed mass of small lymphocytes surrounded by definite ingrowths of fibrous tissue from the adjoining thickened alveolar walls. These ingrowths contain few blood vessels but an abundance of cells from 5 to 10 per cent of which are plasma cells; the rest are small lymphocytes and fibroblasts. The alveolar walls, two to three times normal thickness, contain many small lymphocytes and a few plasma cells, and the alveoli are about one-third filled by large oval cells with eccentric nuclei and faintly granular cytoplasm. A large bronchus has no noteworthy alteration of its cartilage, but the submucosa near this has many dilated blood vessels and few plasma cells; but the latter and also small lymphocytes are abundant about a bronchial gland and near the basement membrane of the mucosa. In places, edematous young fibrous tissue rich in capillaries has bulged the mucosa inward.

In sections of the upper lobe of the left lung bordering on one of the small firm nodules mentioned, the bronchial walls are frayed in many places, and there is considerable desquamation of the lining epithelium. There is an abundance of small lymphocytes in the interalveolar spaces and in the connective tissue surrounding a homogeneous nodule into which a coal-pigmented sprout projects at one point. Here there are also plasma cells, for the most part scattered diffusely, but in places in clumps of from 10 to 30.

In another section of the upper lobe of the right lung (fig. 3 *E*), the alveoli are markedly dilated and thin-walled, so that the nuclei bulge into the lumens of about one-third of them. In other parts of the section, the alveolar walls are broader, especially near the small arteries whose walls are about twice normal thickness and in the fibrous tissue about which there are many small lymphocytes. Bordering on the last described place is a zone of thick-walled alveoli heavily infiltrated with small lymphocytes, and here the bronchioles are dilated. Into this zone run occasional small strands of fibrous tissue, offshoots from whorls and wider strands containing many small lymphocytes, and a few islands of elongated thick-walled alveoli. A mass, staining pink with eosin, borders this place and is about one-fourth sprinkled with small lymphocytes, and contains a few thick-walled blood vessels, mostly centrally, a few alveolar remnants barely distinguishable and a few fibrous and elastic tissue bands peripherally, but no bronchioles.

In a third section of the right upper lobe (fig. 3 *F*) is an abundance of young fibrous connective tissue containing many small lymphocytes, mostly scattered but in places in clumps around the thick blood vessels. Bordering this place is one in which the fibrous tissue becomes more dense, with fewer nuclei and small lymphocytes. This merges with less compact tissue where small lymphocytes are abundant, and from this fibrous sprays pass into a homogeneous mass. In the latter, thick-walled blood vessels and a slight degree of fissuring only are discernible, and small lymphocytes occupy about 5 per cent of the region.

In some places (fig. 4 *J*) the alveolar epithelium is three or four layers thick. Contiguous to such places are collections of small lymphocytes connected with similar masses, surrounding the bronchioles. The internal elastic lamina is the best preserved structure of most of the smaller arteries. The elastic fibers are in places separated by small lymphocytes, and the outermost fibers are frequently broken where there are large collections of lymphocytes in the media. Occasional elastic fibers are in the fibrous tissue generally and pass also into the necrotic places. Fragmented elastic tissue fibers make alveolar outlines perceptible as through a veil in parts of the last mentioned places, in other parts such outlines are reinforced by collections of fibrin. Near many of the gummas fibrin is also abundant in the alveoli.

No tubercle bacilli were found in smears of the bronchial mucus. Stains for spirochetes were not made, as it was thought the optimal time for the demonstration of such organisms had passed before the lungs were thoroughly investigated.

SUMMARY

Acquired pulmonary syphilis with gumma formation is a comparatively rare disease.

The gumma in the lung is in general like the gumma elsewhere, except that in some instances there is a decrease in the width of the peripheral lymphocyte zone.

Spirochaeta pallida has rarely been demonstrated in pulmonary syphilis.

The discrepancy between clinical and necropsy incidence of lung syphilis is due to the more rigid, standardized, histologic criteria used by pathologists.

PATHOLOGIC CHANGES IN VOLUNTARY MUSCLE

II. EXPERIMENTAL STUDY OF DEGENERATION AND REGENERATION OF STRIATED MUSCLE WITH VITAL STAINS *

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In our recent study [1] of degeneration and regeneration of the rectus abdominis muscle in fatal pneumonia following influenza and measles, one of the most interesting problems encountered was the origin of the various cells which compose the "sarcolemmschläuche" of Waldeyer, i. e., the collection of cells within the sarcolemma of the degenerated muscle fiber. These cells, as we have shown, are of two general types, namely, cells which are responsible for the regeneration of the fiber and cells which are responsible for the removal of the hyaline débris resulting from degeneration of the contractile substance. It has been shown that the origin of the regenerative cells is the nuclei and sarcoplasm of the stump of the old fiber, and that these cells may be rightly called muscle cells. The origin of the phagocytic cells, on the other hand, furnishes a more complicated problem. These are pretty clearly of two types: first, those which are unquestionably from the blood stream such as leukocytes and lymphocytes, and, second, cells of a peculiar morphology which does not definitely identify them with any of the usual phagocytic cells of the tissues or of the blood stream. The latter type is the predominating phagocytic element and is typical of the degenerative process as observed in the hyaline degeneration which is followed by regeneration of the fiber. In this paper, I shall discuss principally the origin of these cells as we have observed it in experimentally produced muscle degeneration. I shall also compare the regenerative process as it occurs in animals with that which I have described in man.

Zenker,[2] in his original description of hyaline changes in the rectus abdominis, observed an accumulation of cells in the sarcolemma. He believed that regeneration of the fiber proceeded from some of these cells, but in view of the unusual number in the supporting tissue of the muscle, he was inclined to the view that all the cells within the sarcolemma, both phagocytic and regenerative, were of connective tissue

* From the Department of Pathology, the Johns Hopkins University, Baltimore.

1. Forbus: Pathologic Changes in Voluntary Muscle. I. Degeneration and Regeneration of the Rectus Abdominis in Pneumonia, Arch. Path. **2**:318 (Sept.) 1926.

2. Zenker: Veränderungen der Willkürlichen Muskeln im Typhus Abdominalis, Erlangen, 1863.

origin. Several years later, Waldeyer [3] centered his attention on the cells within the sarcolemma and created the term "Muskelzellenschlauche," which carried with it the opinion of the writer that all the cells, both regenerative and phagocytic, lying within the sarcolemma had their origin from the nuclei of the old muscle fiber. He did not overlook a marked proliferation of cells of the adventitia of the blood vessels and the nerves, but was able to differentiate cells arising from these structures from those within the sarcolemma on the basis of their morphology. Weber [4] suggested the idea that muscle cells might be transformed into "inflammatory cells." This term he apparently applied to the phagocytic cells. All the cells within the sarcolemma he considered derivatives of muscle nuclei. Janovitsch Tschainski [5] injected aniline dyes into the blood stream in animals in which muscle degeneration and regeneration were in progress, and found that many of the cells within the sarcolemma enveloped the dyestuff. He made no attempt on the basis of this observation to differentiate between the different types of cells present, nor would he say that this fact was valid argument either for or against the opinion that the cells within the sarcolemma sheath were derived from "white blood cells;" apparently, however, he did believe that the vitally stained cells were wandering cells. Two years after the appearance of Janovitsch Tschainski's work, Gussenbauer [6] published the results of a study of muscle degeneration and regeneration in which he advanced the opinion that all the phagocytic cells of the sarcolemma sheath were white blood cells which wander in from without the muscle. His chief argument in support of this view lay in the very early appearance of these cells following the necessary injury to the muscle. He believed that insufficient time had elapsed for the formation of such large numbers of "muscle cells." He recognized therefore two distinct cell types, one phagocytic and having its origin outside of the muscle, and the other nonphagocytic and regenerative, and having its origin from the muscle nuclei. Kraske [7] and later Volkmann [8] attributed

3. Waldeyer: Ueber die Veränderungen der quergestreiften Muskeln bei der Entzündung und dem Typhusprozess, sowie ueber die Regeneration derselben nach Substanz-defecten, Virchows Arch. f. path. Anat. **34**:473, 1865.

4. Weber: Ueber die Neubildung quergestreifter Muskelfasern, insbesondere die regenerative Neubildung derselben nach Verletzungen, Virchows Arch. f. Path. Anat. **49**:216, 1867.

5. Janovitsch Tschainski: Ueber die entzündlichen Veränderungen der Muskelfasern. Studien aus dem Institute für experimentelle Pathologie in Wien, 1869.

6. Gussenbauer: Ueber die Veränderungen des quergestreiften Muskelgewebes bei der traumatischen Entzündung, Arch. f. klin. Chir. **12**:1011, 1871.

7. Kraske: Experimentelle Untersuchungen ueber die Regeneration der quergestreiften Muskeln, Habschr., Halle, 1878.

8. Volkmann: Ueber die Regeneration des quergestreiften Muskelgewebes beim Menschen und Sügetier, Beitr. z. path. Anat. u. z. allg. Pathol. **12**:233, 1893.

the origin of all the cells of the sarcolemma to the muscle nuclei. Volkmann divided the muscle cells into two groups, one of which performed the phagocytic function of clearing up the débris of the degenerated fiber, while the other led to restoration of the fiber. More recently vital staining methods again have been applied to the problem of the origin of the cells within the sarcolemma. Kiyono [9] injected carmine into the blood stream during the degeneration and regeneration of the muscle fiber and found that Waldeyer's "Muskelzellenschlauche" was composed of "sarcoblasts"—nonvital staining cells—leukocytes and macrophages, vitally stained. The macrophages were thought to be derived from the surrounding connective tissue. In spite of the failure of the sarcoblasts or muscle cells to take the vital dye, Kiyono was not sure but that some of these cells might be partially responsible for removal of the degenerated contractile substance In the more advanced muscle changes in which budding was frequently observed as a part of the regenerative process, none of the buds were found which had absorbed the vital dye. In Wolbach and Frothingham's [10] description of degeneration and regeneration of the rectus abdominis in pneumonia, the phagocytic cells are considered as of extramuscular origin, and classed as mononuclear wandering cells of the tissues which come into the damaged area, as usually occurs in any ordinary tissue injury. This opinion apparently was based entirely on the morphology of the cells and on the general laws which govern the body reaction to injury.

It is therefore obvious that the exact nature of the phagocytic cells still remains uncertain. With a view to gaining some objective data in connection with the origin of these cells, we have performed the following experiments.

DESCRIPTION OF EXPERIMENTS

Experiment 1.—In order to eliminate as far as possible any idiosyncrasy of any one type of animal in the reaction of its particular muscle to injury, a variety of species was used. Dogs, rabbits, white rats and guinea-pigs were chosen.

The animals were first vitally stained by injection of trypan blue. In the dogs and rabbits the dye was given intravenously, the jugular vein being used in the dogs and the marginal ear vein in the rabbits. With the smaller animals (rats and guinea-pigs), the injections were given intraperitoneally. A 1 per cent solution of trypan blue was used. Sufficient sterility was secured by bringing the solution to a boil and maintaining it at this temperature for one minute. In the injections precautions were taken for asepsis. Daily injections were given until the animals became deep blue. With the dogs a dose of 15 cc. was given the first day, and this was increased to 20 cc. the second day. The latter dosage was continued for five days, at the end of which the animal was sufficiently stained. The rabbits were started with a 5 cc. injection, and this was gradually increased to 15 cc. over a period of five days, at the end of which sufficient stain-

9. Kiyono: Die vitale Karminspeicherung, Jena, 1914.

10. Wolbach and Frothingham: The Influenza Epidemic at Camp Devins in 1918. A Study of the Pathology of the Fatal Cases, Arch. Int. Med. **32**:576, 1923.

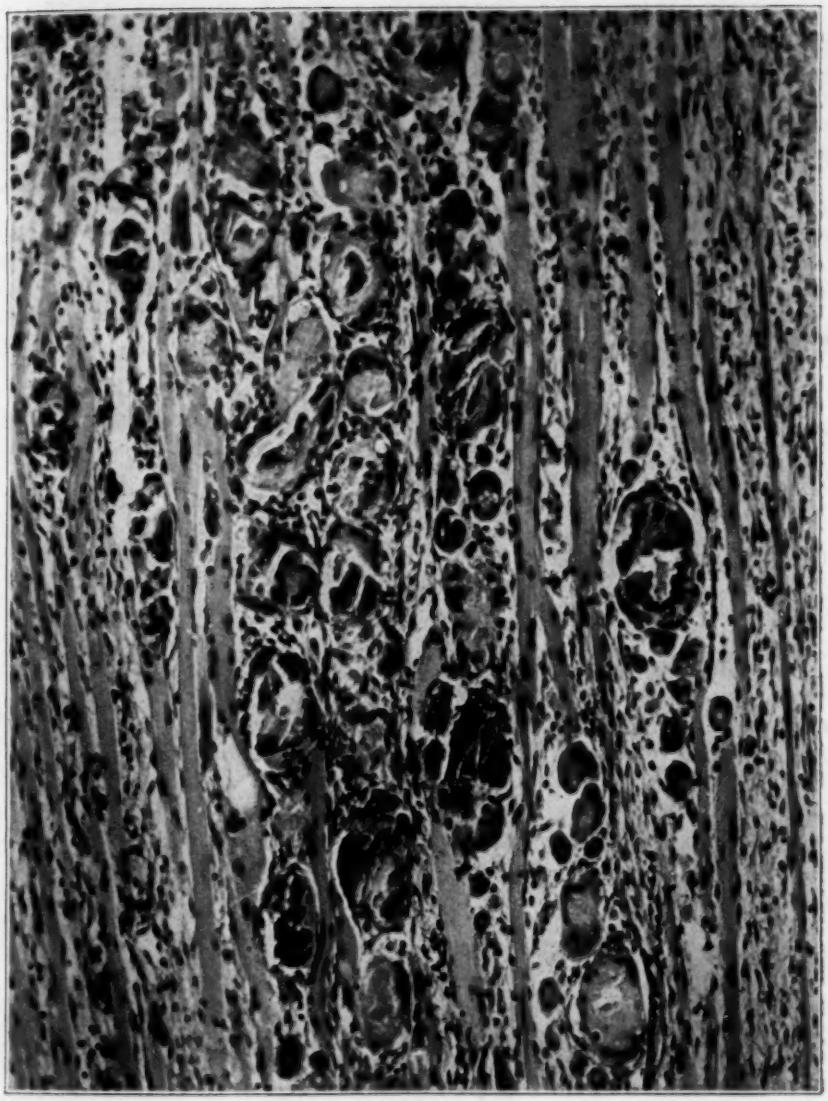


Fig. 1.—Hyaline degeneration of striated muscle of rabbit in edge of zone injected with 95 per cent alcohol. The cells surrounding the hyaline clumps are largely mononuclear wandering cells. A few leukocytes and lymphocytes may be seen. Large syncytial cell masses are conspicuous. A branched fiber is seen near the center of the photograph.

ing was obtained. The rats received 4 cc. doses, the amount not being increased as with the larger animals. Five cc. doses were given the guinea-pigs until sufficient staining was produced.

When an intense staining of all the superficial tissues had been obtained by the injections, the dye was discontinued for a period of five days, thereby allowing sufficient time for clearing of the blood stream of free dye, it being assumed that all the vital dye would be absorbed by the phagocytic cells of the body. Examination of the blood in smears at this time showed no free or absorbed pigment.

At the end of five days, injury was produced in the muscles of the various animals. In the rabbits and dogs one of the larger muscles of the thigh was isolated and ligatures placed so as to stop the blood supply to the whole muscle. The muscle was allowed to remain in this anemic state for three hours. At the end of that time the ligatures were removed and the wound closed. All of the operative procedures were done under aseptic conditions. In the rabbits and guinea-pigs injury to the muscle was produced by injection of phenol dissolved in glycerine.

The muscle lesions thus produced in the previously vitally stained animal were allowed to progress for a maximum period of fourteen days. The injured muscles were excised at intervals and sectioned. The tissues were fixed in Zenker formol and in 10 per cent formalin, embedded in paraffin and cut. Frozen sections were made also from the formalin fixed tissues. Some of the sections were stained with carmine only, others with hematoxylin and eosin, and still others with Van Gieson's connective tissue and muscle stain. It was found that the most satisfactory preparations from the standpoint of the vital staining were obtained with Van Gieson stain.

Experiment 2.—Rabbits alone were chosen for this experiment. The animals were not vitally stained. Injury to the muscles was produced by injection of the phenol dissolved in glycerine. The muscle changes were allowed to progress for twenty-two days, at the end of which time the animal was killed and the damaged muscle extirpated and fixed in 10 per cent formalin and Zenker formol. Sections were cut from paraffin and stained with hematoxylin and eosin, Van Gieson, Mallory connective tissue stain, and Wright and Wilson blood stains.

Experiment 3.—Again rabbits alone were used. Experiment 1 was repeated with the use of carmine [11] for vital staining and with some variation in the method of production of injury to the muscle. Phenolized glycerine was again employed. In addition to this, boiling water and 95 per cent alcohol were used in separate muscles. The muscle changes were allowed to progress for a maximum of eighteen days. The animal was killed and the affected muscles fixed in Zenker formol and in 10 per cent formalin. The sections were cut from paraffin and stained by various methods as mentioned in experiment 2.

Experiment 4.—Rabbits received intramuscular injections with phenolized glycerine, boiling water and 95 per cent alcohol. The muscle changes were allowed to progress for five days. At the end of this time intravenous injections of carmine were given daily for nine days. On the fifteenth day following injury to the muscle and the ninth day of injection of carmine, the animal was killed, and the injured muscle fixed and sectioned as in experiments 2 and 3.

In all the experiments it was found that the rabbits and rats were most resistant to the effects of the vital dye. The trypan blue proved very toxic for the dog. Good staining was produced, but the animal did not live long after

11. A 5 per cent suspension of Grubler's carmine in saturated lithium carbonate, brought to boiling and filtered after cooling.

satisfactory staining was obtained. The rats were found to be highly resistant to the toxic effects of the trypan blue. Large doses of carmine invariably killed the rabbits. By beginning with small doses (2 cc.), however, a deep red was produced, indicative of intense and satisfactory staining of the animal. Both dyes are rapidly excreted in the urine and feces. The color of the tissues begins to fade very soon after discontinuation of the injections.

Experiments 1 and 3 were performed with the purpose of staining all the normally present wandering cells of the animal previous to the production of any

Fig. 2.—High power magnification of wandering cells in degenerating muscle vitally stained with carmine. Rabbit vitally stained with daily injections of carmine. After complete staining, injections stopped for few days, at the end of which injury to muscle was produced by injection of 95 per cent alcohol. The vitally stained cells predominate in the typical field of degeneration as shown in figure 1.

injury to the muscle. Under such conditions the reaction to injury of the muscle could be easily analyzed. With no vital dye available for phagocytosis at the time of injury to the muscle, the presence of vitally stained cells within the sarcolemma sheath of the injured muscle might rightly be interpreted as evidence that the phagocytic cells had wandered in from without. On the other hand, should

no vitally stained cells be found within the sarcolemma, it might justly be assumed that the phagocytic cells had originated from the muscle nuclei and hence were "muscle cells."

We also hoped to be able to differentiate muscle cells from purely phagocytic cells by their difference in reaction to the vital dye. Experiment 4 was performed with this purpose in view.

In experiment 2 we simply wished to study the reaction of the animal muscle to destructive injury.

In the following section results of the experiments are discussed.

MICROSCOPIC STUDY

Degeneration.—On microscopic examination of the experimentally produced degenerated muscle, it is at once apparent that the damage to the muscle is in many ways different from that observed in fatal cases of pneumonia, as we have described, and in typhoid fever as originally described by Zenker,[2] and later by Hoffmann [12] and Volkmann.[3]

At the site of injection of the irritant (phenol, alcohol, boiling water), one finds death of the muscle with complete necrosis of all the fibers, contractile substance, nuclei and sarcolemma, as well as the connective tissue of the muscle. Nothing remains alive at the center of the injected area. It is toward the periphery where one finds only mild damage to the muscle that degenerative changes similar to those present in pneumonia occur.

In this peripheral zone the sarcolemma may persist. If such be the case the contractile substance is found in clumps within this membrane and without striations either longitudinal or transverse. The picture is characteristically that of typical hyaline degeneration as we have described it in detail in our former paper. The muscle nuclei retain their normal staining reaction, and morphologically appear unchanged. It is the contractile substance which suffers the great damage.

The fact that the central part of the injured area becomes potentially a defect in the muscle leads one to expect a repair process which is somewhat different from that observed in typhoid and pneumonia. This indeed proves to be the case.

In summary, one finds that the experimentally produced injury to muscle shows itself as follows (1) necrosis of the central part of the injured area, creating a potential defect in the muscle substance—a destructive lesion; (2) hyaline degeneration of the contractile substance of the fibers in the peripheral zone of the injected area, with or without destruction of the sarcolemma—an irritative lesion.

Preliminary Process of Repair.—The repair process manifests itself first by conspicuous outpouring of cells the purpose of which is to

12. Hoffmann: Ueber die Neubildung quergestreifter Muskelfasern insbesondere beim Typhus abdominalis, Virchows Arch. f. path. Anat., Oct. 5, 1868.

remove from the field of injury all débris resulting from damage to the muscle. In the central zone where the muscle is entirely necrotic, there are wandering cells of many types, leukocytes, lymphocytes, plasma cells, mast cells, clasmatocytes. The mononuclear wandering cells predominate. The leukocytes and lymphocytes are comparatively insignificant in number. In the vitally stained animals the majority of the mono-

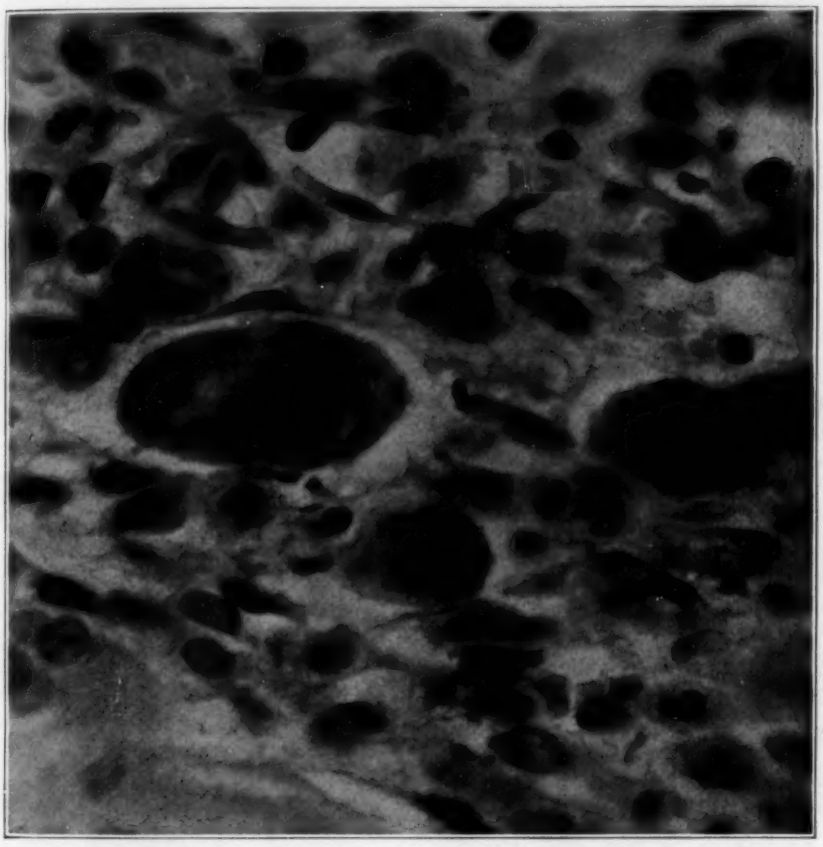


Fig. 3.—High power magnification of vitally stained large syncytial cell masses of muscular origin. The animal from which this section was taken was vitally stained by intravenous injection of carmine. The injury to the muscle produced by injection of 95 per cent alcohol was brought about during the period of administration of the vital dye. The large syncytial cell masses can be shown to be continuous with old, well preserved fibers in serial sections.

nuclear cells contain enveloped granules of carmine or trypan blue, as the case may be. There are some, however, which contain no dye.

In the peripheral zone of injury one sees the cleaning up process in its most florid state. The spaces between the fibers are filled with wan-

dering cells, practically all of which contain enveloped particles of vital dye. This accumulation of cells is especially noticeable along the blood vessels. Some of the cells are obviously on their way into the field of injury, while others containing phagocytized débris are on their way out of the field of operation. The endothelial cells of the blood capillaries are sometimes found loaded with the vital dye, and hence care must be used in differentiating these from the wandering phagocytic cells which lie between the degenerated fibers.

Where the sarcolemma has been left intact, the characteristic "Muskelzellenschlauche" of Waldeyer is found. About the clumps of hyaline contractile substance are grouped large numbers of cells which morphologically are in every way similar to the mononuclear wandering cells which we have just described in the spaces between the fibers and in the central zone of necrosis. It is clear that these cells are phagocytic, for they contain particles of débris within their cytoplasm, as well as numerous particles of vital dye. While it is true that not all of the phagocytic cells within the sarcolemma are vitally stained, it is reasonably certain that the stained and unstained cells are of identical type, for they do not differ from each other morphologically. The other cells within the sarcolemma are those which are concerned with regeneration of the fiber, as can readily be seen by their morphologic character. They contain none of the vital dye, and therefore either are nonphagocytic, or no vital dye has been available during their development. This then is the nature of the "Muskelzellenschlauche," as seen in animals vitally stained five days before any injury is produced in the muscle. To summarize, it may be said that its components are as follows: (1) hyaline contractile substance, without evidence of any striations, in clumps; (2) phagocytic cells vitally stained and not vitally stained, the former predominating; (3) cells morphologically different from the phagocytic cells and unquestionably of muscle origin, not vitally stained.

In view of the fact that in this muscle no dye was available for cells formed after injury to the muscle, it seems reasonable to assume that the vitally stained cells within the sarcolemma must have existed before the injury and therefore are of extramuscular origin, having wandered in from the surrounding tissues.

The fact that there are some unstained phagocytic cells within the sarcolemma must be explained. One may ask why they are not phagocytic cells which arise from muscle cells, and hence for which no vital dye was available, because of the fact that injury to the muscle was produced some days after cessation of administration of the dye. Two facts seem to furnish sufficient argument for the extramuscular origin of these unstained phagocytic cells: 1. Morphologically they are similar to the vitally stained phagocytic cells. 2. Reproduction of the phago-

cytic cells within the sarcolemma is rapid, giving rise to many young cells the cytoplasm of which may contain no vital dye, or so little that it cannot be seen.

In studying the muscle referred to above, it seemed possible to differentiate between muscle cells (regenerative cells) and cells of extra-

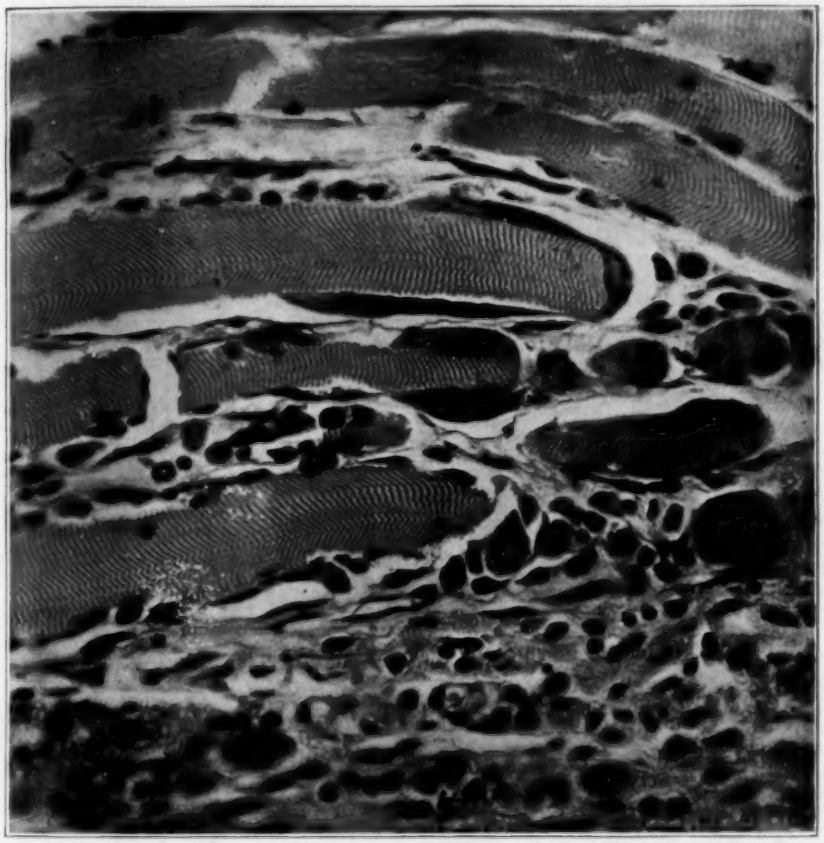


Fig. 4.—High power magnification of experimentally produced hyaline degeneration of striated muscle. One of the fibers still retains its cross and longitudinal striation, but all its nuclei have disappeared except in its sarcolemma. Lying along the side of the hyaline fibers are long spindle cells representing newly developing fibers. Wandering cells and large muscular syncytial cell masses are scattered throughout the field.

muscular origin (phagocytic cells), almost entirely on the basis of the ability or inability of the respective cell to take up the dye. In order to be sure of this, experiment 4 was performed, in which the administration of vital dye was continued during the progress of muscle changes following injury. In tissue removed from these animals, cells which are

morphologically unquestionably of muscle origin are packed with the vital dye. Kiyono states that the regenerative muscle cells were unstained in his preparations which were obtained from experiments similar to ours, but was not sure but that some of these muscle cells might "break loose from the sarcolemma," as he put it, and become phagocytic. There seems to be little question from our experiment that muscle cells may absorb the vital dye if it be available, and that vital staining reaction alone is not sufficient ground for differentiation between muscle cells and phagocytic cells.

Regeneration of Muscle.—Thus far we have considered only the preliminary processes in the repair of the damaged muscle, consisting largely of the removal of the dead material from the site of injury. While the cleaning up of the muscular débris is progressing, through the activity of the wandering cells, the muscle nuclei rapidly reproduce themselves and contribute their share toward the formation of the "Muskelzellenschlauche." This is first noticed in the stump of the injured fiber. The nuclei become surrounded by an abundance of cytoplasm forming muscle cells. These cells may be so packed together that they have the appearance of a syncytium. When vital dye is available, as in experiment 4, these cells may show particles of dye within their cytoplasm. The muscle cells may exist, on the other hand, as single round or spindle shaped elements which, though definite in outline, appear to project from the stump of the injured fiber. When they are so arranged they give to the stump of the old fiber a frayed-out appearance.

In the early stages of the development of the muscle cells in the stump of the old preserved fiber, there appears to be continuity of the cytoplasm of the new cells and the sarcoplasm of the old fiber, the latter being much increased in bulk and apparently serving as the source of the cytoplasm of the new muscle cells. Later, however, the newly formed cells appear to become independent and exist as large syncytial masses of cytoplasm containing numerous nuclei, or as single round or spindle mononuclear cells. Sometimes multinucleated syncytial masses may be encountered just beneath the sarcolemma of the old preserved fiber and quite removed from the stump. This has been spoken of in the literature as lateral budding. One's belief in the existence of a true lateral and terminal budding of old sarcoplasm seems to depend on the interpretation of the pictures just described. In the earlier stages there is undoubted continuity of new cell cytoplasm and old fiber sarcoplasm, but later it seems equally clear that the new cells may exist and develop as individual units. It seems to us more logical to think of the process of regeneration thus described, not as true bud formation, but as a development of new fibers from new cells derived from the nuclei and sarcoplasm of old fibers.

Reproduction of the new muscle cells is by mitosis. This progresses rapidly. The new fiber arises from the muscle cells. A single cell may develop into a new fiber by continuous growth in its cytoplasm accompanied by successive divisions of its nucleus. On the other hand, the new fiber may arise apparently as a result of fusion of many muscle cells while they are still very young, as, for example, at the time when the cells appear in the form of a syncytium. Longitudinal striations appear at a comparatively early stage in the development of the fiber. Cross striations have not been found in any of our sections, which represent muscle changes as old as twenty-two days.

It appears from the presence of fat in the cytoplasm of many of the muscle cells, as well as from the poor staining reaction of the nuclei, that all the newly formed muscle cells do not develop into fibers, but that some degenerate. Should all these cells develop, the size of the regenerated muscle would far exceed that of the original. The defect in the old muscle is never completely filled by new muscle. The greater part of the affected area is filled up by scar tissue in which are embedded isolated and grouped strands of regenerated fibers. Experimental regeneration, it thus appears, is strikingly different from that observed in pneumonia and in typhoid fever, in which regeneration is total, that is, without obvious scar formation.

In connection with the process of repair, we have observed a formation of cartilage in the damaged muscle. This occurred only in the muscle which was injected with alcohol but did not occur in all muscle so treated. The cartilage is present in the inner part of a rather dense scar, the meshes of which are filled with wandering cells. The remains of a few old muscle fibers are seen in the surrounding connective tissue. Some of these show evidence of nuclear proliferation in an attempt at regeneration. Toward the periphery of the scar may be seen damaged muscle in which regenerative changes are progressing. Briefly, the development of this cartilage seems to be about as follows: (1) partial destruction of muscle followed by scar formation with diffuse infiltration of the connective tissue by mononuclear wandering cells; (2) imprisonment of wandering cells in a hyaline stroma; (3) disappearance of cytoplasm of imprisoned cell, with persistence of the cell nucleus; (4) progressive hyalinization of the developing intercellular substance and eventually bluish staining of the hyaline material.

The cells which are imprisoned in the hyaline stroma are (1) round or oval and (2) the size of a large mononuclear cell of the blood; (3) they have a deeply stained, round, eccentrically located nucleus and (4) granular, abundant, vacuolated cytoplasm, stained faintly blue with Wilson blood stain.

The process of development of cartilage does not fall within the scope of our present study, and for this reason we here simply call attention to its presence.

A more detailed description of the regenerative changes in the muscle under discussion does not seem necessary or desirable here in view of the detailed descriptions given of the process of experimental regeneration by many of the older workers, and to which we can add very little. Our chief interest and purpose being the study of the character of the cells which compose the "Muskelzellenschlauche" of Waldeyer, and especially the origin of the phagocytic cells, we are content to confine our efforts to the details of this phase of the study and refer to such admirable works as that of Volkmann,[8] Kraske,[7] Kirby,[13] Neumann,[14] and Nauwerck [15] for the details of experimental regeneration.

SUMMARY

Degeneration of striated muscle was produced in vitally stained animals by intramuscular injection of powerful irritants, such as phenol, 95 per cent alcohol and boiling water, or by cutting off the blood supply for a period of three hours.

Damage to the muscle produced by irritants leads to a necrosis of all the tissue at the center of injection, causing a potential defect in the muscle, and an irritative lesion, hyaline degeneration of the contractile substance of the muscle fibers, at the periphery of the injected zone.

Damage to the muscle is followed by a repair process, consisting of removal of the necrotic material by phagocytic cells and replacement of the destroyed muscle by scar tissue containing some regenerated muscle fibers.

The phagocytic cells found within the persistent sarcolemma, which together with the muscle cells derived from the muscle nuclei form the "Muskelzellenschlauche" of Waldeyer, are wandering cells of extramuscular origin and have no connection with the muscle cells.

Muscle cells, as well as phagocytic cells of the "Muskelzellenschlauche," are capable of being stained by intravenous injection of vital dyes (carmine or trypan blue) during their development, and hence such vital staining alone is insufficient for differentiating between cells of muscular origin and cells of extramuscular origin.

The phagocytic cells found within the sarcolemma can, however, be differentiated from the muscle cells if the animal be first vitally stained

13. Kirby: Experimentelle Untersuchungen ueber die Regeneration des quergestreiften Muskelgewebes, Beitr. z. path. Anat. u. allg. Pathol. **11**:302, 1892.

14. Neumann: Ueber den Heilungs-process nach Muskelverletzungen, Arch. f. mikroskop. Anat. **4**:323, 1868.

15. Nauwerck: Ueber Muskelregeneration nach Verletzungen, Jena, 1890.

and injury to the muscle produced later, after all available dye has been absorbed by the phagocytic cells of the tissues.

Regeneration of the muscle is effected by cells which develop from the nuclei and sarcoplasm of the old preserved muscle fiber. The new fiber appears to arise either from a single muscle cell or from fusion of several such cells.

Cartilage may develop in the scar resulting from injury to muscle by injection of 95 per cent alcohol.

BLOOD DESTRUCTION IN THE MESENTERIC LYMPH GLANDS OF GUINEA-PIGS *

JOHN S. LINGENFELTER, M.D.

DETROIT

The recent studies of Peabody and Broun [1] have shown that in pernicious anemia there is an excessive blood destruction brought about apparently by the phagocytosis of the patient's red blood cells by endothelial cells of his own bone marrow. In a later paper by Broun, Ames, Warren and Peabody,[2] it is pointed out that in certain cases of pneumonia, tropical spru, streptococcus septicemia, hemolytic jaundice and typhoid fever, there is also blood destruction indicated by an increase in the bilirubin content of the blood plasma.

In the light of this knowledge it may be of interest to describe a few observations made on the blood destructive action of reticulo-endothelial cells of the mesenteric lymph glands of guinea-pigs.

While examining histologically the tissues of guinea-pigs which had been used in the production of antirabbit hemolytic serum, a rather unusual picture was disclosed in one of the mesenteric lymph glands. The gland was apparently somewhat exhausted of lymphocytes, the cortex being relatively thin and the medullary cords quite far apart. Within the dilated lymph sinuses occurring uniformly throughout the gland were large mononuclear cells, which gave every appearance of having been derived directly from the lymph gland reticulo-endothelial cells; these cells were completely surrounded by adherent red blood corpuscles. The nuclei of these cells were about 10 mm. in diameter, and were irregularly round, oval or notched, with a well defined membrane, considerable nuclear juice and one or more nucleoli. The cytoplasm of each cell was finely granular, fairly abundant and stained a light pink with hematoxylin and eosin.

Prior to the discovery of this picture, the guinea-pig had been treated in the following manner:

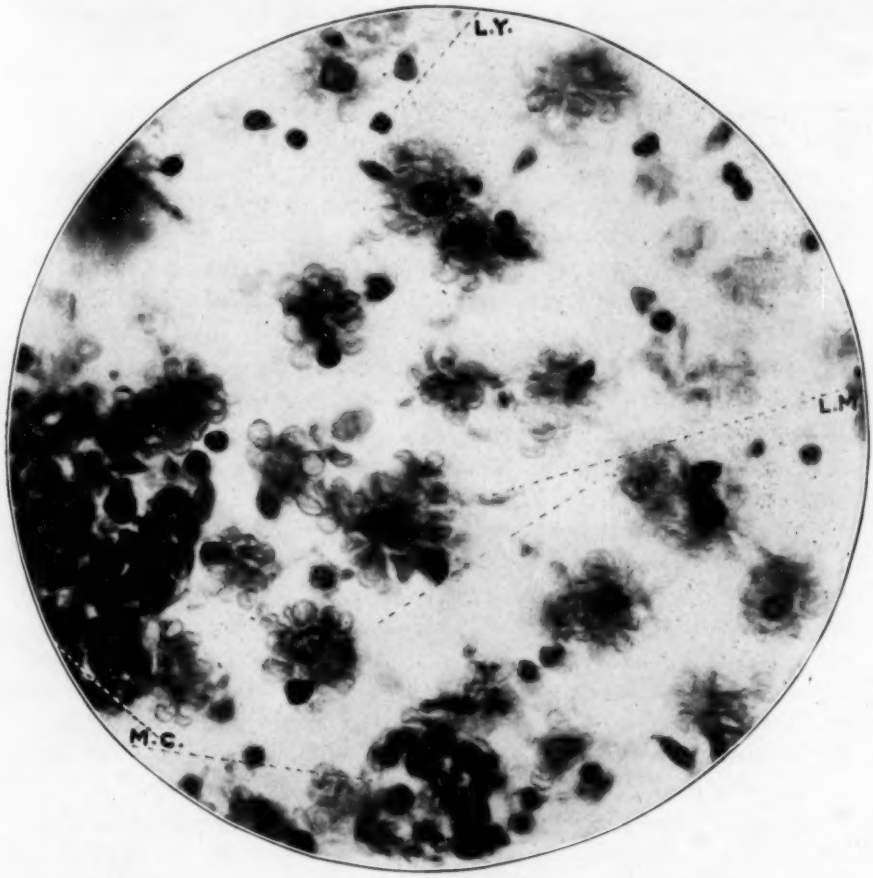
On November 20, 3 cc. of a 50 per cent suspension of washed rabbit red blood cells, diluted in terms of whole blood, had been injected intraperitoneally. On November 22 and 24, respectively, two more 3 cc. injections of red blood cells had been given. On December 1, one week after the last injection of red cells, under ether anesthesia, 5 cc. of blood was drawn from the heart by means of a hypo-

* From the Department of Pathology of the University of Wisconsin.

1. Peabody, F. W., and Broun, G. O.: Phagocytosis of Erythrocytes in Pernicious Anemia: A Preliminary Note, J. A. M. A. **82**:963 (March 22) 1924.

2. Broun, G. O.; Ames, O.; Warren, S., and Peabody, F. W.: Blood Pigments in Pernicious Anemia, J. Clin. Invest. **1**:295-316 (Feb.) 1925.

dermic syringe, and the antirabbit hemolytic titer determined. The hemolytic titer was found to be very low (less than 500 units per cubic centimeter, so on December 2, 4 and 6, the animal was again given intraperitoneal injections of washed rabbit red blood cells, the dosage being as before. On December 15 the animal was bled to death (under ether), the tissues examined grossly, and fixed in Zenker's solution. The serum was again titrated against rabbit red blood cells, and this time a somewhat higher, though still quite low, titer was obtained—750 units per cubic centimeter.



Mesenteric lymph gland of guinea-pig that had beeen immunized to rabbit red blood cells; × 600. *M.C.* indicates medullary cords of lymphocytes; *Ly.*, lymphocytes; *L.M.*, large mononuclear cells with adherent red blood corpuscles.

The gross examination of the tissues revealed nothing abnormal except a slight increase in lymphatic tissue, but histologic examination disclosed the picture described above in one of the mesenteric lymph glands.

A pancreatic lymph node also showed essentially the same picture. Other lymph glands were not noticeably abnormal except that a few

cells with red blood corpuscles adherent to them could be found in some of the lymph sinuses.

This rather striking lymph gland picture raised two questions in particular: Could it be that these red blood cells, which were so abundantly agglutinated about the mononuclear cells, were actually remnant from antigenic rabbit cells, which had been injected into the peritoneal cavity nine days before the animal was killed? A negative answer to this question would leave but one origin for these cells—the blood of the guinea-pig itself. It seemed hardly possible that the adherent red blood cells could have been antigenic, especially since the guinea-pig's serum had caused complete hemolysis of a unit of rabbit cells in a dilution of about 1 to 750, but Bergel [3] had described an agglutination phenomenon which seemed to suggest that the red blood cells might have been antigenic. He stimulated the formation of an exudate by the injection of sheep cells into peritoneal cavities of mice. Then under the microscope he observed an agglutination of sheep red cells about "nongranular mononuclear" cells which he obtained from the exudate.

One other guinea-pig treated precisely as the one described above, was killed and examined. At necropsy this animal gave the same gross observations as the former, that is, nothing abnormal was observed except the increase in lymphatic tissue, but histologic examination of the mesenteric lymph glands disclosed a slightly different condition. Here, the gland, while not so exhausted of lymphocytes as the former, yet contained in its lymph sinuses a considerable number of reticulo-endothelial cells, a few of which possessed adherent red blood corpuscles; but the large majority of them were filled with hemosiderin. Apparently there had either been phagocytosis of the red cells previously by these cells, or else red cells, which had previously been adherent to them, had been hemolized, and the hemoglobin absorbed into the cytoplasm and oxidized to hemosiderin.

In order to determine whether or not the red cells which had been observed were actually antigenic cells, or whether they had come from the guinea-pig's own circulation, a series of experiments was performed in which chicken red blood cells were injected intraperitoneally into guinea-pigs, in single and repeated doses, and the animals killed and examined histologically after various intervals of time. Chicken red cells were chosen for antigen, since, as they are nucleated, they could easily be differentiated histologically from the guinea-pig's own cells. Normal guinea-pigs were killed and examined histologically as controls.

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3. Bergel, S.: Beitrage zur Lehre von der Hämogglutination und Hämolyze, Ztschr. f. Immunitätsf. Exper. Therap. **27**:44-458, 1918.

EXPERIMENTS WITH CHICKEN CELLS AS ANTIGEN

In the first experiment, a guinea-pig was given a single intraperitoneal injection of 3 cc. of a 50 per cent suspension of washed chicken red blood cells, and the animal was killed twenty-four hours later. Smears were made from the lining of the peritoneal cavity, and the tissues were fixed in Zenker's solution. Careful histologic examination of mesenteric lymph glands, omentum, spleen, liver and other organs, as well as smears from the peritoneal cavity, revealed no evidence of chicken red blood cells, but in a pancreatic node were found many cells like those described in the beginning of this paper. But the adherent red blood cells were *not* chicken cells. Some of them were well preserved, and showed no trace of a nucleus, and therefore they must have come from the guinea-pig's own circulation. This observation practically answered our first question. The adherent red cells observed in the first guinea-pig lymph gland were not necessarily antigenic, for here was found the same condition, and in this case it could be certain that the adherent red blood cells were not those which had been injected into the animal. Then, too, this and subsequent experiments showed the rapidity with which foreign red cells, when injected into the peritoneal cavity of a guinea-pig, are destroyed. In no case did the foreign red cells remain unhemolyzed in the peritoneal cavity, or in the lymph glands, for more than a few hours. So, in view of the fact that the last dose of red cells had been given nine days prior to death, it did not seem possible that the adherent red cells observed in the first guinea-pig could have been antigenic.

Histologic examination of the tissues of guinea-pigs which had been repeatedly injected intraperitoneally with washed chicken red cells uniformly showed a hyperplasia of the endothelial cells of the mesenteric lymph glands. Some of these cells possessed adherent red cells, some contained red cells within their cytoplasm, and some contained hemosiderin as evidence of blood destruction. As a rule, no chicken cells could be demonstrated within the mesenteric lymph glands.

However, in the case of an animal that had died, spontaneously, subsequent to its fourth intraperitoneal injection of chicken red cells, the lymph sinuses of one of the mesenteric lymph glands contained a great many chicken cells, many of which were packed within phagocytes. Here, too, in the mesenteric lymph glands in which no chicken cells were found, there was a definite hyperplasia of endothelial cells, indicating that, as a reaction to the intraperitoneal injection of foreign red cells into the guinea-pig, there is a hyperplasia of these phagocytes in the mesenteric lymph glands.

In another experiment, a guinea-pig was immunized to chicken red blood cells by three intraperitoneal injections given at two and four day intervals. Then after an interval of eight days, the guinea-pig was

given a rather large dose (5 cc. of a 50 per cent suspension) of chicken cells intraperitoneally, and the animal killed an hour later. On examination, clumps of chicken cells were found free in the peritoneal cavity and adherent to the omentum. In these clumps, as well as in the adjacent omentum, were many large mononuclear cells similar to those observed in the lymph glands. Many of these cells contained chicken cells, and some contained granules of hemosiderin. In this case no chicken cells could be found in the mesenteric lymph glands, although here too, there seemed to be a definite increase in the number of reticulo-endothelial cells.

Although chicken cells had been observed within the endothelial cells of the lymph glands, we were not convinced that here, too, as in the case of the animal's own red cells, phagocytosis might not be preceded by a stage of adhesion of the red cells to the phagocytes. The difficulty in observing this, following the intraperitoneal injection of chicken cells, lay in the fact that many of the cells injected into the peritoneal cavity were hemolyzed, or engulfed by phagocytes, before reaching the lymph glands, and, after reaching the lymph glands, the stage of adhesions of the red cells to the phagocytes must have been brief, if indeed such a stage occurred at all. To avoid this difficulty, and to observe the earlier stages of the phagocytosis of foreign red cells by the endothelial cells of the lymph glands, two guinea-pigs, one immunized against chicken cells, and one normal, were anesthetized with ether, and small doses of chicken cells were injected directly into the mesenteric lymph glands. One-half hour later the animals were killed, and the lymph glands immediately fixed in formol-Zenker's solution. In one of the lymph glands so treated many chicken cells were observed well within endothelial cells. Others could be found, adherent to, but not yet engulfed by, these same endothelial phagocytes. From this it seems that foreign red cells, as well as red cells from the guinea-pig's own blood, adhere to, before they are engulfed by, endothelial cells of the mesenteric lymph glands. It seems that for foreign red cells the stage of adhesion is much briefer in time than it is for the animal's own cells.

CONTROLS

The mesenteric lymph glands of three normal guinea-pigs were examined as controls. In two of these animals evidence of blood destruction was fairly well marked. Endothelial cells containing blood pigment were frequently observed, and a few were found with red blood cells adherent to them. In one animal, although a fairly large number of endothelial cells were observed in the lymph sinuses, no evidence of blood destruction could be found.

Obviously more normal lymph glands should be examined before conclusions are drawn, but it seems fairly certain that red cell destruction

is at least a potential function of the mesenteric lymph glands of guinea-pigs even if it cannot be observed in every case.

In this connection must be mentioned the observation of a lymph gland which essentially duplicated the picture that first aroused our interest. The animal was not normal, but had received no injections of red cells. This guinea-pig had just been received in the laboratory in a rather poor lot of stock animals, and was first noticed lying in the pen, emaciated and weak. The animal was killed. Examination of the organs showed an acute bronchitis, and areas of bronchopneumonia, which, with probable undernourishment, was sufficient cause for the condition of the animal. However, in the lymph spaces of all the mesenteric lymph glands were found large numbers of red blood cells adherent to endothelial cells, several sections being essentially a duplication of that of the first gland described in this paper.

COMMENT

That the endothelial cells of the spleen and hemolymph nodes, when the latter exist, function in destroying red blood cells, has long been accepted, but that the mesenteric lymph glands also function in this manner has not received much emphasis, even though it is generally recognized that the cells lining the sinuses of all lymph nodes are actively phagocytic for all foreign cells brought to them through the afferent lymphatics. Pearce, Krumbhaar, and Frazier [4] observed that, while the number of endothelial cells in the lymph glands of normal dogs was not great, yet these cells not infrequently contained blood pigment and an occasional red cell. After splenectomy the number of endothelial cells increased markedly, but the phagocytosis of red cells did not appear in any greater degree than in nonsplenectomized dogs. However, when these animals received injections of hemolytic serum, and when they lived long enough after the injections, the mesenteric lymph glands assumed the function of red cell phagocytosis in a marked degree. Karsner, Admiral and Bock [5] confirmed these observations in cats, discovering abundant phagocytosis of red cells in the lymph glands of splenectomized animals that had received hemolytic serum, but not observing red cell phagocytosis in the lymph glands of normal cats. However, it seems that since the lymph glands so readily exhibit this function in time of need, most probably red cell destruction is a function normally exercised in a slight degree. This function seems to be increased by the injection of foreign red blood cells into the

4. Pearce, R. M.; Krumbhaar, E. B., and Frazier, C. H.: The Spleen and Anemia, Philadelphia, J. B. Lippincott Co., 1918, pp. 164-180.

5. Karsner, H. T.; Admiral, H. H., and Bock, A. V.: A Study of the Influence of Splenectomy and of Certain Organs and Organ Extracts on Hemopsonins of the Blood Serum, J. M. Res. **30**:383-391, 1914.

peritoneal cavity. In certain other conditions also, as, for example, in the animal with bronchopneumonia, this red cell destroying function seems to be increased in a marked degree.

It is also interesting to note that this red cell destruction is not carried on by immediate phagocytosis, but that preceding phagocytosis there is an apparent adhesion of the red cells to the endothelial cells. At present we cannot be certain that phagocytosis within the mesenteric lymph glands takes place at all in the destruction of the guinea-pig's own red cells. It may be that the red cells are hemolyzed on the surface of the endothelial cells, the hemoglobin being absorbed into the cytoplasm. However, when foreign red cells are introduced directly into a lymph gland they are rapidly and completely taken within endothelial cells, but even here the phagocytosis is apparently preceded by a short transitory stage of adhesion to the phagocytes. When foreign red cells are introduced into the peritoneal cavity of a guinea-pig, those that are not hemolyzed while free in the cavity or are not destroyed by phagocyes, are in part conducted into the lymph spaces of the mesenteric lymph glands to which they adhere, and are then destroyed within endothelial cells.

As to whether or not this red cell destruction, when stimulated to excess, may be a factor in the mechanism of the production of anemia, remains to be proved. As mentioned in the beginning of this paper, Peabody and his co-workers have pointed out that in pernicious anemia there is an excessive red cell destruction, the mechanism of which seems to be a phagocytosis of red blood cells by the endothelial cells of the bone marrow. Apparently the same mechanism plays a part in the anemia accompanying certain infections. The explanation may be that as a reaction to infection there is a hyperplasia of these red cell destroying phagocytes, or, as seems more probable, there may be some hemolytic toxin in the blood stream, injuring red cells, and thereby rendering them more susceptible to phagocytosis. This increased demand for red cell destroying phagocytes may in turn stimulate a hyperplasia of such cells in the spleen, lymph glands and bone marrow.

The bacterial analogue of the adhesion of red corpuscles to phagocytes was observed in vitro by Rosenow in 1906.[6]

SUMMARY

1. The mesenteric lymph glands of guinea-pigs normally function to a greater or lesser degree in the destruction of red blood cells.

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6. Rosenow, E. C.: The Rôle of Phagocytosis in the Pneumococcocidal Action of Pneumonic Blood, J. Infect. Dis. **3**:683, 1906.

2. This destruction is apparently accomplished by an adhesion of the red cells to reticulo-endothelial cells, which adhesion may precede phagocytosis.

3. Foreign red blood cells injected into the peritoneal cavity of the guinea-pig, are conducted in part into the lymph sinuses of the mesenteric lymph glands, where they are taken up by reticulo-endothelial cells, phagocytosis being preceded by adhesion of the red cells to the phagocytes.

4. The injection of foreign red blood cells into the peritoneal cavity of the guinea-pig apparently stimulates a hyperplasia of reticulo-endothelial cells, phagocytic, or adhesive to, the animal's own red blood cells.

MEDIASTINAL CARCINOMA OF TRACHEAL ORIGIN

REPORT OF A CASE

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AND
CLARENCE KUMMER, M.D.
BUFFALO

The following case of mediastinal carcinoma of tracheal origin is reported because of the comparative rarity of the condition.

REPORT OF CASE

History.—G. H., aged 36, unmarried, a foreman in an automobile factory, came under observation in June, 1925, complaining of a swollen neck. He said that the night before he could close his collar as usual, while that morning there was a gap of 4 inches between the ends of the collar. Once before his neck was swollen for a short time. For the past month he had been moderately short of breath, although he had worked every day and had played baseball three evenings a week. These were his only complaints, and he made very light of them.

He had had measles, mumps and chickenpox when a child and diphtheria when 6 years old.

His father died of cancer of the bladder at the age of 61, his mother of cerebral hemorrhage at 65. Four brothers and one sister were alive and well, and one brother and one sister had died in infancy. One uncle died of cancer of the liver. There was no history of venereal disease, and the patient was a man of exemplary habits.

Physical Examination.—The patient was robust and muscular with a rather thick neck. His face was slightly cyanotic, and the superficial veins of his neck and upper part of the chest were prominent. There was slight dyspnea, and his voice was rather husky. Over the upper right side of the chest anteriorly was an area of dulness extending from the midline about 4 inches to the right. Over this area respiratory sounds were diminished. The heart was not found enlarged on percussion, and the apex beat was within the nipple line.

The thyroid gland was palpable in the neck and not enlarged. Lymphatic glands were not palpable in the neck or axillae. The temperature was 99 F., the pulse rate 80 and respirations 20.

Roentgen-ray examination confirmed the suspicion of a mediastinal tumor (fig. 1). Owing to the smooth outline of the tumor, as shown by the roentgen ray, it was hoped that it might not be malignant. The patient was given roentgen-ray treatment for about four weeks, but without improvement.

The patient was operated on, July 27, under local anesthesia. The tumor was hard and smooth and extended medially under the sternum and laterally under the pleura. Hemorrhage from one of the perforating arteries caused the termination of the operation.

On July 29, again under local anesthesia, the second costal cartilage and part of the sternum opposite the second and third costal cartilages up to the midline were removed. It was impossible to strip the pleura from the tumor, so it was incised. The tumor extended in all directions and was tightly wedged in among the structures in the superior mediastinum, making removal of the tumor impossible.

The patient died twelve hours after the operation.

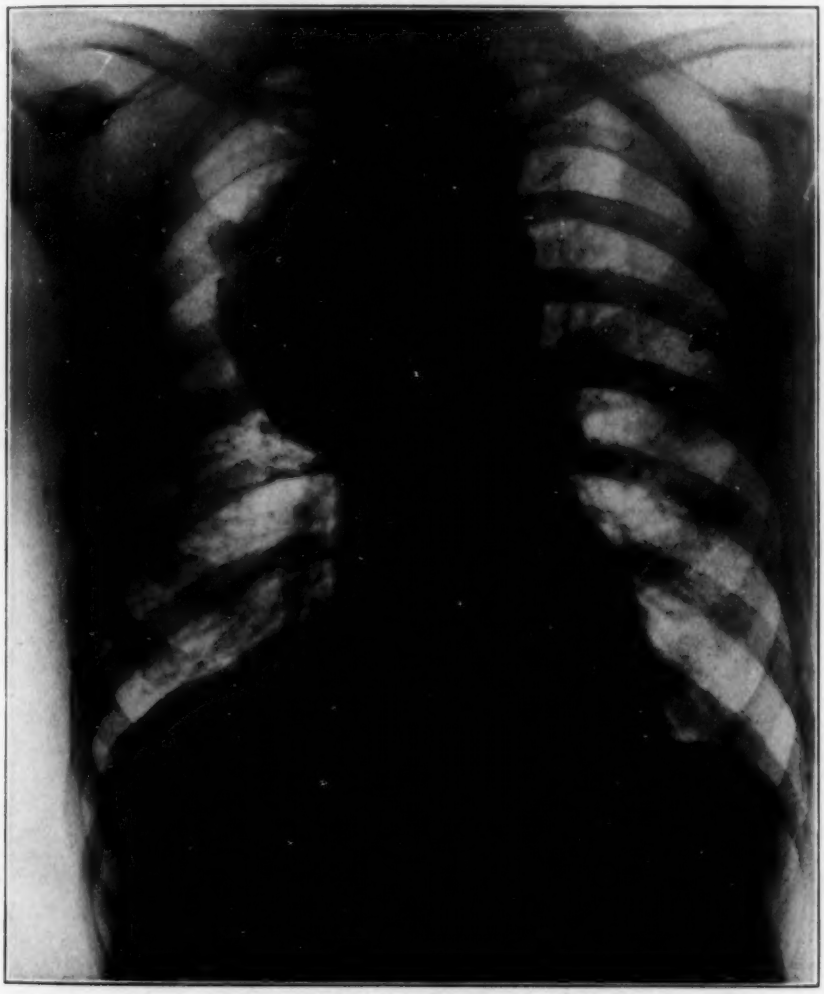

Fig. 1.—Roentgenogram of mediastinal carcinoma.

Necropsy.—This was performed by Dr. Theodore Mueller. He found marked rigor mortis. On the thorax anteriorly and to the right, in the region between the first and fourth ribs, there was a rectangular wound.

The second and third right costal cartilages had been resected and also about one-half inch of the sternum in that region. On removing the sternum, both

pleural cavities were found free from adhesions and exudation. In the upper mediastinum there was a large tumor mass to which the median side of the right lung was adherent. The contents of the thorax were taken out in toto.

The left lung showed marked hypostasis. The right lung showed an old calcified and encapsulated tuberculous process in the upper lobe measuring about one-half inch in diameter. The trachea was then opened from behind (fig. 2). The mucosa of the trachea was reddened and injected. At the bifurcation there were

Fig. 2.—Pathologic condition of trachea.

several areas in which the cartilage and mucous membrane had been replaced by whitish hard tumor masses. There was also a small area of ulceration.

A large, hard, nodular tumor filled the whole upper mediastinum and had destroyed all bronchial and mediastinal lymph nodes. It had perforated into the posterior wall of the pericardium. The weight of the tumor was three-quarters of a pound (0.3 Kg.).

The heart and the large vessels apparently were not affected. The heart was flabby and dilated. The valves were intact. Microscopic examination of sections showed a mucous membrane epithelioma.

Pathologic Diagnosis.—Tracheal carcinoma with metastases in the bronchial lymph nodes; old healed tuberculosis of the upper part of the right lung.

COMMENT

Cases of pulmonary carcinoma are being reported in much greater numbers than formerly. Adler in 1912, as quoted by Ewing,[1] tabulated 374 cases, and Scott, in 1922, reported 120 cases. Holzer[2] reports a great increase of bronchial carcinoma in Central Europe. At the General Pathological and Anatomical Institute in Prague, the percentage of bronchial to other cancers has increased 300 per cent. The cause of this increase is not known. There are many possibilities. In a recent editorial[3] of *The Journal of the American Medical Association,* these are discussed, among those mentioned being irritation from smoking cigarets with inhalation of smoke, inhalation of dust, precancerous changes in the lungs due to tuberculosis and influenza, tarring of our highways and inhalation of irritating gases from automobiles and imperfect fuel combustion. Ewing gives tuberculosis as the chief cause. He also mentions trauma and anthracosis, and quotes Wolf as giving the rupture of anthracotic nodes into the bronchi as the cause of bronchial carcinoma

In the case reported, the patient was an intemperate smoker of cigarets. Furthermore, the smoke inhaled would probably cause most irritation at the bifurcation of the trachea, because here it impinges on the mucous membrane with greater force before being deflected into the bronchi.

It is true that the patient had an old calcified tuberculosis in his upper lung, but the carcinoma did not spring from that part of the lung.

According to Ewing's classification, pulmonary carcinoma has its origin from one of the following three structures: (*a*) from epithelium of trachea or bronchi, usually at or below the bifurcation; (*b*) from the mucous glands of the bronchi, usually limited to walls of the bronchi; (*c*) from the pulmonary alveoli. This case belongs to the first class, and Ewing shows a photograph from Langhans to illustrate this class, which is almost similar to figure 2.

Operation was performed in this case in the hope that the tumor might not be malignant. The smooth outline, as shown by the roentgen ray, and the absence of loss of weight and palpable glands supported this hope. Although this was primarily a pulmonary cancer, originating

1. Ewing: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1922, p. 807.

2. Holzer, Hedwig: Med. Klin. **21**:1235, 1925.

3. The Recent Increase in Pulmonary Cancer, editorial, J. A. M. A. **86**:486 (Feb. 13) 1926.

from the trachea, it nevertheless gave all the symptoms of a mediastinal tumor, because it did not invade the pulmonary alveoli and did invade the mediastinal structures. This explains the absence of the pulmonary symptoms, such as cough, expectoration and hemoptysis. Even if a correct diagnosis had been made, operation for the purpose of decompression would probably have been performed. The infiltration of the growth into the mediastinal structures, however, prevented relief from any decompression operation on the chest wall.

THE SOURCE OF GLYCOGEN IN TUBERCLES*

MAX PINNER

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Since Ehrlich's first communication on the iodophile substance in leukocytes, much work has been done on the distribution of glycogen in normal and pathologic tissues. The first reference to tuberculosis is found in a paper by Langhans,[1] who briefly mentions that he never found glycogen in tubercles. In elaboration of Ehrlich's statements, Katsurada,[2] Best [3] and several other investigators report the increase of glycogen in all inflammatory foci. Gierke [4] found glycogen in the outer borders of tubercles with central necrosis in both polymorphonuclear and mononuclear cells, independent of the localization of the tubercle. The occurrence was more marked in acute than in chronic processes. He conceives the glycogenic infiltration as a manifestation of increased metabolic activity. Lubarsch [5] found in most tuberculous products no glycogen, only occasionally in epithelioid cells and leukocytes and once in a giant cell; most frequently in young tubercles from ten to twenty days after infection in guinea-pigs. Still younger tubercles from four to six days after infection were always free from glycogen. Devaux [6] studied the question again in experimental tuberculosis. According to this author, the necrotic foci are always free from glycogen, but the cellular borders of necrotic foci contain a considerable amount of glycogen in leukocytes and epithelioid cells. No glycogen was found in lymphocytes. Tubercles without a central necrosis never contained glycogen. In cells which had engulfed tubercle bacilli more glycogen was found on an average. W. and M. Pagel [7] found the exudate cells in caseous pneumonia always free from glycogen; they found, as did the earlier investigators, glycogen in the cellular borders of tubercles, especially of the exudative type. Much of the glycogen was extracellular but always in definite relation to cellular detritus. The intracellular

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1. Langhans: Virchows Arch. f. path Anat. **120**:28, 1890.

2. Katsurada: Beitr. z. path. Anat. u. z. allg. Pathol. **32**:193, 1902.

3. Best, F.: Beitr. z. path. Anat. u. z. allg. Pathol. **33**:585, 1903.

4. Gierke, von E.: Beitr. z. path. Anat. u. z. allg. Pathol. **37**:502, 1905; Ergebn. d. Pathol. **11**:871, 1907.

5. Lubarsch, O.: Virchows Arch. f. path, Anat. **183**:188, 1906

6. Devaux, C.: Beitr. z. path. Anat. u. z. allg. Pathol. **41**:596, 1907.

7. Pagel, W., and M.: Virchows Arch. f. path. Anat. **256**:629, 1925.

glycogen was seen in mononuclear round cells and in leukocytes. Arndt [8] made his studies on domesticated animals, and reported essentially the same results as the previous workers.

Summarizing the experience of the various workers, one finds agreement on the point that not too young tubercles contain glycogen in some epithelioid cells, sometimes in giant cells and rather regularly in leukocytes. That emigrated leukocytes which wander to an inflammatory focus always contain a considerable amount of glycogen, is a well established fact. It is, however, unexplained as yet why epithelioid and giant cells sometimes contain glycogen and why the same cell types more frequently do not contain this substance; and secondly, what the source of the deposit of glycogen in these cells is. In an attempt to solve these questions the following work was undertaken.

METHODS

A series of rabbits was infected intravenously with bovine bacilli of low virulence and killed six, seven, eight, nine, ten, twelve, fourteen, seventeen and twenty days after the infection. Some tuberculous organs of guinea-pigs were examined thirty days after infection, and some human material from necropsies. A series of guinea-pigs received 0.5 cc. of old tuberculin subcutaneously thirty days after infection; they were killed from five to six hours after the injection of tuberculin, when they exhibited all the signs of a severe tuberculin shock.

Small pieces of tissue, not thicker than 3 mm., were fixed either in absolute alcohol or, according to Neukirch,[9] in a saturated solution of glucose in 40 per cent formaldehyde. Since the latter method of fixation prevents better the diffusion to one side of the cell, it was used exclusively in the course of these studies. After embedding in celloidin, sections from 5 to 6 microns thick were cut and stained by Best's [10] carmine stain.

RESULTS

In very young tubercles, about up to the eighth or tenth day after injection, no glycogen was found. In older tubercles, glycogen is seen regularly in the leukocytes of the outer zone. At the same time, and increasingly so with the age of the tubercle, glycogen appears in epithelioid cells as small droplets and occasionally in giant cells. In these serial examinations it was easy to follow the appearance of glycogen in the last two cell types: whenever there is phagocytosis of a glycogen containing leukocyte, its glycogen appears as a small droplet in the phagocyte. The complete development can be followed; a complete and unaltered leukocyte is engulfed in an epithelioid cell; only the pyknotic fragments of a leukocytic nucleus and a drop of glycogen are seen in the phagocyte (fig. 1); the nuclear fragments disappear and only the

8. Arndt: Klin. Wchnschr. **41**:39, 1925.

9. Neukirch, P.: Virchows Arch. f. path. Anat. **200**:73, 1910.

10. Best, F.: Ztschr. f. wissensch. Mikrosk. **23**:319, 1906.

Figure 1

Figure 2

Fig. 1.—Liver tubercle in a rabbit, seventeen days after infection by the intravenous route, showing a young giant cell containing pyknotic nuclear fragments of a polymorphonuclear leukocyte and a drop of glycogen. Celloidin sections stained by Best's alkaline carmine.

Fig. 2.—Splenic tubercle in a guinea-pig killed during a severe tuberculin reaction thirty days after infection. Around the caseous center is a red zone of glycogen containing polymorphonuclear leukocytes which immigrate into the focus during the tuberculin shock. Celloidin sections stained by Best's alkaline carmine.

glycogen remains as evidence of the phagocytic activity of the cell. How strictly the presence of glycogen is dependent on the emigrated leukocytes is well demonstrated in tubercles from guinea-pigs which were killed in tuberculin shock. The marked leukocytic infiltration of the cellular zones in this stage is shown in the carmine stained section by a bright red ring of glycogen containing leukocytes (fig. 2). The necrotic center of tubercles is usually free from glycogen; sometimes small amorphous granules are seen.

The fact that epithelioid cells frequently envelop leukocytes and digest them until to all appearances nothing is left but their glycogen, shows significantly the vital activity of these cells in tubercles, and is a strong argument against that conception which represents epithelioid cells as devitalized or degenerated forms of reticulo-endothelial or monocytic cells.

Livers studded with tubercles seem to contain less glycogen in their parenchymatous cells than a normal organ, but a definite statement cannot be made to this point without a careful control of the alimentary condition. It was rather regularly observed that the liver cells bordering on necrotic tubercles contained much more glycogen than similar cells further removed from the foci. No explanation for this occurrence is suggested.

CONCLUSIONS

Glycogen appears in tubercles when leukocytes immigrate; it is found in epithelioid cells and in giant cells whenever they engulf leukocytes; and the occasional droplets of glycogen in these cells seem to be the remains of the digested leukocytes. Warkany [11] found that dried tubercle bacilli contain 4.1 per cent of glycogen. The possibility must be considered that small amounts of glycogen may be derived from digested bacilli. Devaux's statement that cells containing tubercle bacilli show a tendency to glycogen deposits may lend some support to this concept. It is obvious that only a minimal part of the glycogen found in tubercles can be accounted for by this occurrence.

SUMMARY

Glycogen is found in tubercles either in leukocytes, or in epithelioid or giant cells. The latter derive their glycogen from leukocytes that have undergone phagocytosis.

By their marked phagocytic and digestive action on leukocytes, the epithelioid cells in tubercles manifest that, though transformed cells, they have retained their functional vitality.

I wish to express my thanks to Dr. R. H. Jaffe, under whose direction this work was done.

11. Warkany, J.: Ztschr. f. Tuberk. **42**:184, 1925.

THE SITE OF FORMATION AND SOURCE OF BILIRUBIN *

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The bile secretory function of the liver is the best known function of this great organ, although probably no better understood than some of its other functions. While the bile has several constituents, the pigment, although it may not be the most important of these, has received the most attention. This is not only due to its striking color and staining qualities, but also to the recognition that it bears a relation to the pigment in the red blood cell and that the clinical condition of jaundice is due to its retention within the body.

Our interest in the formation of bilirubin was elicited as the result of some of our observations in connection with an investigation of the general problems associated with the physiology of the liver. We found that when the liver was totally removed from a dog, a pigment which gave all the reactions for bilirubin accumulated in the plasma, urine and fat. The attempt to learn more about this pigment led to the series of studies which I shall summarize. It will readily become evident, as I describe the various experiments and their results, that it would be impossible for one person to execute all the procedures of such a research. Only through the cooperation of those with special training has research such as this been possible. In this work I was first associated with Magath and later with Bollman and Sheard. In presenting this summary of a group research,[1] I am but the spokesman for my associates.

LITERATURE ON THE SUBJECT

While it is not necessary to review the numerous studies that have been made of jaundice, the formation of bilirubin and the constituents of

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* From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

* Annual address before the Minnesota Pathological Society, Minneapolis, March 16, 1926.

1. Bollman, J. L.; Mann, F. C., and Magath, T. B.: Studies on the Physiology of the Liver. VIII. Effect of Total Removal of the Liver on the Formation of Urea, Am. J. Physiol. **69**:371-392, 1924. Mann, F. C.; Sheard, C., and Bollman, J. L.: XI. The Extrahepatic Formation of Bilirubin, ibid. **74**:49-60, 1925. Mann, F. C.; Sheard, C.; Bollman, J. L., and Baldes, E. J.: The Site of the Formation of Bilirubin, ibid., pp. 497-510. The Formation of Bile Pigment from Hemoglobin, ibid. **76**:306-315, 1926. XIII. The Liver as a Site of Bilirubin Formation, ibid **77**:219-224, 1926.

bile (because that has recently been done by Rich,[2] Whipple,[3] Rous,[4] and Pearce, Krumbhaar and Frazier [5]), it will be of value to emphasize some of the important historic phases of the subject in order to provide a perspective for the more recent work.

Since the pigment was found in the secretion from the liver, it could logically be supposed that it was made in that organ. Virchow,[6] from the results of his studies on the changes occurring in old blood extravasations, raised the question both in regard to the origin of bilirubin from the hemoglobin and the possibility of its being formed outside the liver. The first experiments dealing with the effect of hepatectomy on the production of bile pigment outside the liver were inconclusive because the species employed, the frog, does not produce much bile pigment, particularly during the quiescent period, and the methods for detecting this substance were crude at that time.

The first experimental work which influenced the physiologic concept of bile pigment formation and the clinical concept of jaundice was that of Minkowski and Naunyn.[7] They removed the livers from hens, ducks and geese, species in which such an operation is anatomically possible, and could subsequently find only traces of bile pigment in the urine. They considered that the slight amount of pigment found was that which had been excreted into the intestine before hepatectomy and later absorbed into the blood. They found that jaundice did not occur in the dehepatized fowl following the administration of arseniuretted hydrogen, although it was readily produced in the normal fowl. McNee [8] repeated the experiments of Minkowski and Naunyn on geese and obtained similar results. Whipple and Hooper [9] studied the jaundice that develops in dogs following the injection of hemoglobin, and found

2. Rich, A. R.: The Formation of Bile Pigment, Physiol. Rev. **5**:182-224, 1925.

3. Whipple, G. H.: The Origin and Significance of the Constituents of the Bile, Physiol. Rev. **2**:440-459, 1922.

4. Rous, Peyton: Biliary Aspects of Liver Disease, Am. J. Med. Sc. **75**: 625-631, 1925.

5. Pearce, R. M.; Krumbhaar, E. B., and Frazier, C. H.: The Spleen and Anemia, Philadelphia, J. B. Lippincott Company, 1918.

6. Virchow, R.: Die pathologischen Pigmente, Arch. f. path. Anat. u. Physiol. **1**:379-404, 1847.

7. Minkowski, O., and Naunyn, B.: Ueber den Icterus durch Polycholie und die Vorgänge in der Leber bei demselben, Arch. f. exper. Path. u. Pharmakol. **21**: 1-33, 1886.

8. McNee, J. W.: Experiments on Haemolytic Icterus, J. Path. & Bact. **18**: 325-342, 1913-1914.

9. Whipple, G. H., and Hooper, C. W.: Icterus. A Rapid Change of Hemoglobin to Bile Pigment in the Circulation Outside the Liver, J. Exper. Med. **17**: 593-612, 1913.

that dogs with an Eck fistula developed jaundice similar to that in normal dogs under the same conditions. The diversion of the portal blood and decrease of the blood supply to the liver did not present or delay the occurrence of icterus, although it was diminished. They concluded that the liver could not be the essential factor in the conversion of hemoglobin to bilirubin. In another series of experiments they ligated the aorta and vena cava just caudal to the diaphragm and obtained an accumulation of pigment in the blood and fat which gave a positive reaction for bilirubin. Mann and Magath [10] noted the constant observation of icterus in dogs that had survived total removal of the liver for more than six hours. The plasma, urine and fat became definitely yellow with a pigment that gave a positive test for bilirubin. Rich [11] repeated Whipple and Hooper's experiments and was not able to corroborate the results. McNee and Prusik [12] were likewise unable to confirm the results of Whipple and Hooper in the majority of their experiments. Bickel [13] and Makino,[14] employing the method of removal of the liver described by Mann,[15] noted the development of jaundice in the totally dehepatized animals. Rich [16] in another series of experiments demonstrated the presence of bilirubin in the plasma of dogs in which he had removed all the intra-abdominal viscera.

McNee concluded from the results of his study of the experimental production of icterus in birds that the polygonal cells of the liver were not responsible for the making of the pigment but that the Kupffer cells were the important agents. As these cells belong to the reticulo-endothelial system described by Aschoff,[17] he considers that bile pigment is made by these cells which are found mainly in the liver, spleen and bone marrow. This theory that the reticulo-endothelial system is the site of formation of bilirubin has received support from the work

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10. Mann, F. C., and Magath, T. B.: The Effect of Total Removal of the Liver, Tr. Section Path. & Physiol., Am. Med. Assn., 1921, pp. 29-42.

11. Rich, A. R.: Experimental Studies Concerning the Site of Origin of Bilirubin, Bull. Johns Hopkins Hosp. **34**:321-329, 1923.

12. McNee, J. W., and Prusik, B.: The Effect of Experimental Exclusion of the Liver on the Formation of Bile Pigment, J. Path. & Bact. **27**:95-110, 1924.

13. Bickel, A.: Leberexstirpation und Avitaminose in ihren Beziehungen zum Zuckerstoffwechsel, Deutsche med. Wchnschr. **39**:140-141, 1923.

14. Makino, J.: Beiträge zur Frage der anhepatocellulären Gallenfarbstoffbildung, Beitr. z. path. Anat. u. z. allg. Pathol. **72**:808-859, 1924.

15. Mann, F. C.: Studies on the Physiology of the Liver. I. Technic and General Effects of Removal, Am. J. Med. Sc. **161**:37-42, 1921.

16. Rich, A. R.: On the Extrahepatic Formation of Bile Pigment, Bull. Johns Hopkins Hosp. **36**:233-247, 1925.

17. Aschoff, Ludwig: Lectures on Pathology, New York, Paul B. Hoeber, 1924.

of Lepehne,[18] who attempted to prevent the formation of bilirubin by blocking these cells with other substances, and also Eppinger [19] and Elek.[20] Schilling,[21] Rosenthal and Melchior,[22] Rosenthal and Fischer,[23] and Bieling and Isaac [24] were not able to show that blocking the reticuloendothelial cells with other substances decreased the formation of bile pigment. Melchior, Rosenthal and Licht [25] studied the development of bile pigment following removal of the liver and following the injection of toluendiamin into normal and dehepatized dogs. They found that bilirubin increased in the plasma of the dehepatized dog, but that the jaundice following the injection of toluendiamin was prevented by the removal of the liver. They concluded that while extrahepatic formation of bilirubin is possible, it is insignificant in amount, and that the liver is essential for the development of real jaundice.

While there have been many contradictions in the data presented and conclusions drawn in regard to the relation between hemoglobin and bile pigment from the time of the original observations of Virchow,[6] Herrmann,[26] Tarchanoff [27] and Stadelmann [28] to those of the most recent investigators, Rich [29] and Ernst,[30] the evidence is conclusive that bilirubin

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18. Lepehne, Georg: Milz und Leber. Ein Beitrag zur Frage des hämatogenen Ikterus, zum Hämoglobin—und Eisenstoffwechsel, Beitr. z. path. Anat. u. z. allg. Pathol. **64**:55-127, 1918. Untersuchungen über Gallenfarbstoff im Blutserum des Menschen, Deutsches Arch. f. klin. Med. **132**:96-120, 1920. Weitere Untersuchungen über Gallenfarbstoff im Blutserum des Menschen, ibid. **135**:79-107, 1925.

19. Eppinger, Hans: Die hepato-lienalen Erkrankungen, Berlin, Julius Springer, 1920.

20. Elek, Ladislaus: Experimentelle Untersuchungen über das retikulo-endotheliale System, Klin. Wchnschr. **3**:143-147, 1924.

21. Schilling, Viktor: Die angebliche Rolle der Sternzellen im Bilirubinstoffwechsel, Berl. klin. Wchnschr. **58**:881-882, 1921.

22. Rosenthal, F., and Melchior, E.: Untersuchungen über die Topik der Gallenfarbstoffbildung, Arch. f. exper. Path. u. Pharmakol. **94**:28-51, 1922.

23. Rosenthal, F., and Fischer, M.: Ueber die Grundlagen der Lehre vom retikuloendothelialen Ikterus, Klin. Wchnschr. **1**:2265-2269, 1922.

24. Bieling, R., and Isaac, S.: Untersuchungen über die Bedeutung von Milz und Leber für die Entstehung des hämolytischen Ikterus, Verhandl. d. deutsch. Gesellsch. f. inn. Med. **34**:50-53, 1922.

25. Melchior, E.; Rosenthal, F., and Licht, H.: Untersuchungen am leberlosen Säugetier, Arch. f. exper. Path. u. Pharmakol. **107**:238-259, 1925.

26. Herrmann, Max: Ueber den Einfluss der Blutverdünnung auf die Secretion des Harns, Arch. f. path. Anat. u. Physiol. **17**:451-463, 1859.

27. Tarchanoff, J. F.: Zur Kenntnis der Gallenfarbstoffbildung, Arch. f. d. ges. Physiol. **9**:329-334, 1874.

28. Stadelmann, Ernst: Zur Kenntniss der Gallenfarbstoffbildung, Arch. f. exper. Path. u. Pharmakol. **15**:337-363, 1882.

29. Rich, A. R.: The Formation of Bile Pigment from Haemoglobin in Tissue Cultures, Bull. Johns Hopkins Hosp. **35**:415-516, 1924.

30. Ernst, Z.: Untersuchungen über extrahepatogene Gallenfarbstoffanbildung an überlebenden Organen. II, Biochem. Ztschr. **157**:30-38, 1925.

is made from hemoglobin. When free hemoglobin is made available in the normal animal, the production of bile pigment is increased. An increase in blood destruction is followed by an increase in the formation of bile pigment, although the relation is not quantitative.

In this connection, the great importance of the van den Bergh [31] test for bilirubin in the blood should be emphasized. Without the development of an accurate and sensitive test for bilirubin, research on the pigment would have been difficult and the specific clinical studies on jaundice impossible.

EXPERIMENTAL WORK BY THE AUTHOR

As previously stated, our attention was first focused on the formation of bile pigment in relation to our studies on the dehepatized dog. In observing the various changes that took place when the liver was totally removed, we noted that a specimen of urine obtained from the dehepatized animal shortly after operation had a tinge identical with the color when the urine contained bile. The plasma became tinged with yellow between three and six hours after operation. The sclerotics of animals that lived sixteen hours or more after operation became tinged with yellow and an icteric tint could sometimes be seen in the mucous membranes. At necropsy all the fatty tissue was also a dirty yellow and a chloroform extract of this fatty tissue was also yellow. This yellow pigment, wherever found, in urine, plasma and fat, gave a positive reaction for bilirubin by all the chemical tests that we employed, although we placed the most confidence in the van den Bergh test. This test was first indirect, and later sometimes became biphasic and rarely direct. It was also found that the pigment accumulated in the body following hepatectomy, if the spleen was removed either at the same time or previous to the removal of the liver. The pigment also accumulated when all the abdominal viscera were removed, and also when there was no free blood in the peritoneal or pleural cavities.

There appeared to be four possible sources of the pigment: (1) squeezed from the liver at the time of operation; (2) absorbed from the bile present in the intestine previous to operation; (3) stored in some organ previous to operation and (4) made by some other tissue. In these experiments, there was no possibility that the pigment was formed in residual hepatic tissue since all the liver was removed.

That the pigment was not squeezed from the liver at the time of operation is proved by its absence from the plasma immediately after operation, its progressive increase and the failure of careful clamping of the vena cava before hepatectomy to prevent its occurrence. That it

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31. Van den Bergh, A. A. H.: Der Gallenfarbstoff im Blute, Leiden, S. C. van Doesburgh, 1918.

was not just an absorption of bile from the intestine which had been discharged previous to hepatectomy is proved by the fact that removal of the gastro-intestinal tract at the time of hepatectomy did not prevent its occurrence, and that the contents of the gastro-intestinal tract usually became almost pigment-free within a few hours after hepatectomy, while the pigment in the plasma continued to increase progressively. That it had not just been stored in some organ previous to hepatectomy is evident, since the amount excreted in the urine and the amount found in the plasma and tissues at death were too large to have been stored in any one organ or tissue without giving marked evidence of its existence. We are therefore forced to the conclusion that the pigment is made by some organ or tissue without the intervention of the liver. As the pigment appears in animals in which both the liver and spleen are removed, the latter organ is shown to be unnecessary for its formation. Also it appears when no blood is present in the peritoneal or any other serous cavity, so that the cells lining these cavities are not necessary for its production.

The results of our experiments on the dehepatized dog definitely proved that a pigment which presented all the characteristics and chemical reactions of bilirubin accumulated in the urine, plasma and fat. This seemed definite and conclusive evidence that the liver is not essential for the formation of bile pigment. However, the accepted tests for bilirubin are not considered absolutely specific as a few other substances will give a positive reaction with the same reagents, but in general these substances are not normal constituents of the blood. The possibilities were also considered that there may be substances intermediate between hemoglobin and bile pigment as found in the bile which would give positive reactions with the chemical tests for bilirubin. It appeared possible that these intermediate substances are formed outside the liver, but that the elaboration of the pigment in its final form occurs only in the liver. Since we had employed all the chemical methods without overcoming these objections, which, although not of as much importance as they might seem, prevented the positive statement that bile pigment was formed about the liver, we turned to other methods of identification of the pigment. Only the physiochemic method of identification remained. Fortunately, through the cooperation of Sheard, we were able to employ the most recent development in this method to our problem, the spectrophotometer.

It is not necessary to describe in detail the spectrophotometer, the method of employing the instrument or the criteria necessary for interpreting the results, as that has been done previously.[32] It is sufficient to

32. Sheard, C.; Baldes, E. J.; Mann, F. C., and Bollman, J. L.: Spectrophotometric Determinations of Bilirubin, Am. J. Physiol. **76**:577-585, 1926.

assert that with the spectrophotometric method, when properly employed, it is possible to identify and measure accurately minute amounts of bilirubin. It is possible to detect accurately by this method the presence of bilirubin in plasma to one-fiftieth of the smallest amount measurable by the van den Bergh method. In order to illustrate the sensitiveness of the method, it should be noted that in most of our experiments we were dealing with quantities of bile pigment varying from 0.04 to 0.015 mg. for each 100 cc. of blood.

As determined by the spectrophotometer, the curves of light transmission of the pigment which accumulates in the plasma of dehepatized dogs; of animals with all their intra-abdominal viscera removed; of animals with obstructed common bile duct and gallbladder removed and of the pigment in the bile obtained from the gallbladder, were identical in nature. These data proved that the pigment which appears in the urine and blood and accumulates in the fat of the totally dehepatized dog is bilirubin. The extrahepatic formation of bilirubin was a proved fact.

It was known for several years that the normal serum of many species of animals contained yellow pigment. It has only been relatively recently known, however, that in the majority of instances the yellow pigment was bilirubin. The plasma of some species of animals is clear, and no bilirubin can be detected by the chemical methods. This is true of the plasma of the dog. However, the employment of the spectrophotometer for the identification and measurement of the bilirubin in the plasma showed that the colorless plasma of the normal dog contains a small but measurable amount of bilirubin. This fact furnished us with the means of determining further the site of formation of the bilirubin which accumulates in the dehepatized dog.

Since bilirubin accumulated progressively in the body after the liver had been eliminated, it was clear that some organ or tissue, other than the liver, was passing the pigment into the circulation. In an attempt to determine what tissues were responsible for this addition of the pigment to the blood, we measured and compared the bilirubin content of the blood going to the various organs and tissues with that of the blood draining away from the same organs or tissues. An increase in the bilirubin content of the blood leaving a definite vascular area over the content of the blood entering such an area would prove that bilirubin had been added to the blood while it was passing through that particular vascular area. This is a method that has been employed frequently in experimental work in an endeavor to determine what happens to a specific substance in a particular organ. Usually the method has been of questionable value because the change, if such occurred, was usually too slight in relation to the volume of blood flow to be measured by existing methods. However, the spectrophotometric method was so

exact in regard to the identification and determination of minute variations in the amount of bilirubin that it seemed feasible to employ it.

Accordingly, specimens of blood were secured from the major arteries and veins of the body and an estimation of their bilirubin content made. The specimens of blood from the artery and its accompanying vein were obtained almost synchronously, the venous specimen being secured slightly in advance of the arterial specimen in order not to interfere with the blood supply to the part until the venous specimen had been obtained.

The results of these experiments were definite and striking: The bilirubin content of the blood returning from the head, limbs, trunk and spleen was greater than that of the blood going to these structures; the bilirubin content of the blood returning from the kidneys and intestine was the same as that of the blood going to these structures. Blood obtained from the various large arteries of the body contained approximately the same amount of bilirubin. It was evident that bilirubin had been added to the blood as it passed through the spleen and through the extra-abdominal vascular areas. The extra-abdominal vascular areas in which bilirubin was added to the blood contained mainly three kinds of tissue, skin and fascia, muscle and bone marrow. It was proved by the following observations that the bone marrow was the tissue responsible for adding the bilirubin to the blood. When the bone was removed from a hind limb and the circulation maintained to the muscle, skin and fascia, the difference in the bilirubin content of the venous and arterial blood to the limb disappeared. Venous blood obtained directly from the muscle and skin had the same concentration of bilirubin as arterial blood. The bilirubin content of the venous blood from the bone marrow was greater than the arterial blood.

These observations definitely proved that the bile pigment which accumulated in the body following total removal of the liver was formed in the spleen and bone marrow. While the amount of bilirubin formed extrahepatically appeared to be a goodly portion of the total amount formed in the body, the data did not do more than indicate in an indirect way the possible importance of the liver in the formation of the pigment. The question of whether or not any bile pigment was made in the liver, and if so, the relative amount, remained unanswered.

The liver presented several obstacles to the investigation of its importance as a site for the formation of bile pigment. These difficulties are mainly three: first, it has a double blood supply to one of which bilirubin is constantly being added; second, it excretes the pigment under investigation; third, it drains its venous blood through several separate openings into a large vessel where it is readily mixed with blood coming from other vascular areas. These difficulties made it seem problematic

that we would be able to determine definitely whether bilirubin is made in the liver.

However, we found that if the spleen were removed, the amount of bilirubin in the portal blood decreased at once to the same value as that in the arterial blood. It was thus possible, by simply removing the spleen, to make the bilirubin content of the two sources of blood to the liver equal. While the blood from the liver drained from the organ by several veins, we found that by making a cava pocket (produced by temporarily ligating the vena cava between the point of entrance of the lumbosuprarenal vein and the most caudal hepatic vein) it was possible to obtain venous blood from the liver unmixed with blood from other sources. If the blood specimen was secured by passing the needle just cephalic to the ligature, it would seem as though it would consist entirely of hepatic vein blood. However, in some experiments, in order to be certain that respiratory movements had not forced the blood from above into the cava pocket, we also occluded the cava above the diaphragm while taking the blood specimens. Finally, by ligating the common bile duct and removing the gallbladder, it was possible to prevent the loss of bilirubin from the organ. An examination of the bilirubin content of the blood returning from the liver, at a time when the bilirubin content of the arterial blood was increasing following the obstruction to the biliary outflow, would indicate whether or not bilirubin was made in the liver. As the bilirubin content of all arterial blood is the same, the specimens of arterial blood were removed from the femoral artery instead of the hepatic, because we wanted to avoid all possibility of interfering with the blood supply to the liver.

Our first interesting observation in this series of experiments was in regard to the rapidity with which the bilirubin accumulated in the blood following obstruction to the biliary outflow. In some experiments a measurable increase in the bilirubin content of the arterial blood was found within fifteen minutes after removal of the gallbladder and ligation of the common bile duct. In all experiments, a measurable increase was found within thirty minutes after biliary obstruction. It should be emphasized, as noted in another paper,[33] that it is necessary to remove the gallbladder at the time of obstruction of the duct if immediate bilirubinemia is desired. The activity of the gallbladder will prevent the accumulation of bilirubin in the body for many hours after obstruction of the common bile duct.

A comparison of the bilirubin content of the arterial blood and the blood from the hepatic veins, at the time the bilirubin content in the

33. Mann, F. C., and Bollman, J. L.: The Relation of the Gallbladder to the Development of Jaundice Following Obstruction of the Common Duct, J. Lab. & Clin. Med. **10**:540-543, 1925.

former was increasing following the obstruction of the biliary outflow, showed that there was a small but definitely larger amount of bilirubin in the hepatic vein blood than in the arterial blood. Without a doubt a small amount of bilirubin had been added to the blood as it traversed the liver.

The evidence was then complete that bile pigment was made in the bone marrow, spleen and liver but not made in demonstrable amounts in other tissues from which the unmixed venous blood could be obtained. It can readily be surmised that bilirubin might be formed in other isolated areas, particularly in those containing cells of the reticulo-endothelial type, but the total amount of pigment normally formed in these areas must be small. While there could be no question that bilirubin was made in the bone marrow, spleen and liver, the relative amount made in each of these three major sites of bilirubin formation was difficult to estimate. The fact that the bile pigment accumulates in the body of an animal in which both liver and spleen have been removed at approximately the same rate as in a normal animal in which the gall-bladder has been removed and the common bile duct ligated, would appear to indicate that most of the bilirubin is normally formed outside the liver and spleen. The great difference in the bilirubin content of the blood from the splenic artery and vein as compared with the difference in the bilirubin content of the blood from the hepatic artery and hepatic vein, would seem to indicate that more bilirubin is formed in the spleen than in the liver. It would thus appear that while some bilirubin is undoubtedly formed in the liver, the relative amount made in this organ as compared with the total amount made in the whole body is insignificant. The bone marrow would seem to be the most important site of formation of bilirubin.

Most investigators of the subject agree that hemoglobin is a source of bilirubin and, so far as can be determined, the only source. The facts that bilirubin was formed in localized sites, that it could be so readily identified and that slight differences in amounts could be so easily estimated, afforded us the opportunity to determine whether the formation of bilirubin in such localized areas could be increased by making hemoglobin more available. While the experiments were performed with similar results with both spleen and bone marrow, the former, because of the ease with which blood specimens could be secured and hemoglobin injected, was the site chosen for investigation. The procedures were as follows: The spleen was exposed and carefully protected. The vasa brevia and their accompanying arteries were ligated. One of the largest of the ligated veins was exposed and a cannula inserted pointing toward the spleen. The artery accompanying the vein was dissected free. A loose ligature was passed around the splenic vein. By gentle traction on this ligature, blood from the spleen was made to

drain from the cannulized branch and could be collected directly into a centrifuge tube. Hemoglobin was injected directly into the small artery that accompanied the cannulized vein. In this manner, specimens of venous blood could be obtained directly from the spleen and hemoglobin injected directly into the arterial system of the spleen without injuring, to any great extent, the circulation of the organ. Since the bilirubin content of the arterial blood is practically the same, the arterial blood specimens were usually obtained from the femoral artery rather than the splenic because of the danger of injuring the vessel to the spleen if they had been obtained from the latter. The hemoglobin was prepared from washed, laked red cells after the usual manner.

The results of the experiments were definite. Within thirty minutes after the injection of hemoglobin into the splenic artery, there was a definite increase in the bilirubin content of the blood returning from the spleen as compared with that of the blood from the same source before injection, and as compared with the arterial blood. The increase in bilirubin formation would reach a maximum from one and a half to two hours after injection of the hemoglobin and then decrease to the preinjection level.

One of the more interesting observations made in this series of experiments was the fact that as the formation of bilirubin increased following the injection of hemoglobin into the arterial circulation of the spleen, a substance other than bilirubin appeared, reached its maximal concentration at the same time as the formation of bilirubin and decreased as the concentration of bilirubin decreased. This new substance appeared to be hematin.

These experiments proved conclusively that when hemoglobin was injected into the arterial circulation of the spleen, the bilirubin content of the blood in the splenic vein increased. In control experiments in which unhemolized red cells were injected, no such increase in bilirubin occurred. Furthermore, another substance, probably hematin, appeared as an intermediary substance between hemoglobin and bilirubin. Evidently bilirubin is made from hemoglobin in the spleen.

COMMENT

At present the data on bilirubin as a pure substance are meager. Our knowledge of the pigment is derived mainly from studies in which it was mixed with other substances. The objection might thus be made that the pigment that we have been investigating is not bilirubin but a closely related substance. This objection cannot now be eliminated entirely. However, it should be emphasized that the results with the spectrophotometer leave no doubt that the pigment normally found in the gallbladder, the pigment that gives rise to the jaundice following

the obstruction of the biliary outflow, the pigment that accumulates in the dehepatized animal, the pigment that is found in larger amounts in the blood returning from certain vascular areas (such as the bone marrow and spleen) than in the blood going to these areas and the pigment that is increased in the body following the injection of hemoglobin, is one and the same pigment.

It is impossible to determine at present what bearing the results of these studies may have in regard to the conception of jaundice. It is unquestionably true that, as regards the sites of formation of bilirubin, the liver need not be considered of much importance, but nevertheless this great organ must always be directly or indirectly involved in every case of jaundice.

Laboratory and Technical Notes

ELECTRIC HEATING AND CONTROL MECHANISM FOR BACTERIOLOGIC INCUBATORS*

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The electric current as the source of heat to maintain the temperature of the bacteriologic incubator has many advantages. A number of makers supply electrically operated incubators that give satisfactory results. It is often desired, however, to convert an incubator originally intended for gas heating to electric operation, or to secure apparatus that will meet the special requirements of an incubator room. It is necessary in such cases to provide both a heating element and a thermostat for the control of the heating current. The thermostatic devices manufactured especially for laboratory use, however, have been found in the author's experience to be wanting in ruggedness and reliability; but by making use of types of instruments designed primarily for industrial purposes it has been possible to meet the necessary requirements of accuracy and dependability. Apparatus similar in principle to that described here has been utilized by White and Nye,[1] and is in use in some of the larger bacteriologic laboratories. In applying such instruments to the needs of this laboratory, it is believed that an advance has been made both in the design of the instruments and in the method of application, and a brief description may simplify a similar installation.

The apparatus consists of three elements: the thermostat, the heating unit and the relay which delivers current to the heating unit under control of the thermostat. The thermostat comprises a heat sensitive bulb which is placed within the culture chamber of the incubator and an instrument head which is mounted beside the relay on a panel outside the incubator and secured to the wall or to the supporting frame of the incubator. A single connection to the 110 volts direct current lighting circuit is the only source of electric current required.

Two types of thermostat have been employed. The more sensitive form was developed for this application by the Brown Instrument Company of Philadelphia in cooperation with me. The heat sensitive copper bulb ⅛ x 4 inches is connected by flexible capillary tubing 6 feet in length with a Bourdon tube in the instrument head. This tube,[2] arranged in the form of a helix, expands with rise in temperature of the methyl chloride contained within the system and moves an arm affixed to the free end of the helix without the intervention of multiplying devices. The arm is pivoted; its free end moves across a graduated scale between 25 and 50 C. and indicates the temperature

* From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

1. White, B., and Nye, R. N.: A Bacteriological Incubator Room, J. Lab. & Clin. Med. **9**:790, 1924.

2. The cross-section of this tube is flattened or oval. With increase in internal pressure the volume of the tube increases by an approach to circular cross-section. This results in expansion of the coil in which the tube is disposed. The principle is that employed in the familiar pressure gage.

of the bulb. Adjustable contact arms are situated one on either side of the pointer arm. When the pointer arm moves above the temperature desired it makes contact with the "high" arm and closes the circuit between the *C* and *H* binding posts on the outside of the instrument; when the pointer moves below the temperature desired, the "low" arm makes contact with the pointer, and the circuit between posts *C* and *L* is closed. These circuits are carried into the relay, which is operated by them, opening or closing the heavier circuit through one portion of the heating unit. A resistance in the relay permits only a very small current to pass from the lighting circuit through the delicate contacts in the thermostat. The bulb of the thermostat is carried through one of the ventilating ports in the side of the incubator shell, and is secured in the upper part of the culture chamber, remote from the heating unit and from the doors. The flexibility of the capillary tubing permits the placing of the bulb and tubing in any desired location; the bulb may be placed directly in a basket of culture tubes of particular importance. Control is thus carried out through the air temperature of the culture chamber; the advantage of this method over control of the water-jacket temperature has been pointed out by Clark.[3] The space corresponding to 1 C. on the scale over which the pointer moves is about 8 mm. in length, and the contact arms can be adjusted to give a range of movement of the pointer arm of ± 0.1 C. from any desired point. The lag usually associated with the vapor-tension principle employed in this instrument is minimized by the small size and heat capacity of the bulb, so that the actual variations in the temperature of a tube of culture medium are less than those of the bulb.

The second type of thermostat used likewise possesses a sensitive bulb, connected by capillary tubing with an expanding coil in the instrument head. It was adapted to the present application and produced by the Motometer Co., Long Island City, N. Y. This instrument employs the gas expansion principle; the bulb is larger and has a greater heat capacity than that of the first type described. It is somewhat less sensitive than the first instrument, but has a smaller instrument head and is lower in cost.

The relay is a type used for the remote control of electric current.[4] It is mounted on the instrument panel beside the thermostat head. The relay possesses six terminals, which for convenience in installation are best connected by short wires to large insulated binding posts of the radio type, on the panel. The proper connections are easily made between these binding posts and the lighting circuit, the heating unit and the thermostat posts. The advantage of this relay is that when the "low" circuit through the thermostat is closed, this circuit is at once shunted in the relay, and fluttering of the contact points does not cause sparking; when the "high" circuit is closed in the thermostat, it is immediately broken by the release of the armature in the relay, and cannot be closed again until the "low" circuit has operated. The relay will safely break a current of 15 amperes at 125 volts. The heavy current contacts are durable and require no attention.

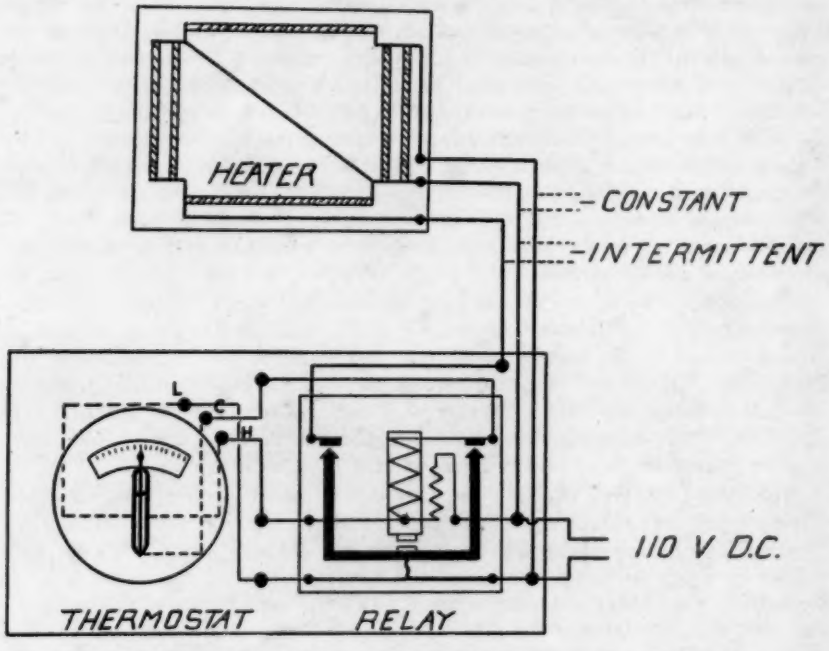
The heating unit for the individual incubator is a type in common use in the laboratory.[5] It is composed of two plates of asbestos board, between

3. Clark, W. M.: The Determination of Hydrogen Ions, ed. 2, Baltimore, Williams & Wilkins Company, 1922.

4. Manufactured by the Signal Engineering and Manufacturing Co., New York City.

5. Manufactured by the Central Scientific Co., Chicago.

which are mounted six resistance-heating coils. The wiring of the unit as furnished by the manufacturer has been altered so as to make two separate circuits, each of three coils connected in parallel. One of these circuits is connected directly to the lighting circuit and is in constant operation; by itself it maintains the temperature of about 6 cubic feet of culture space between 30 and 35 C. during the winter months. In summer this maintenance circuit is reduced to two coils. The other circuit is closed intermittently by the thermostat through the relay. The more closely the maintenance circuit meets the heat requirements of the incubator (without at any time exceeding them) the smaller may be the heating capacity of the intermittent circuit. This means less frequent operation of the thermostat and relay contacts and more constant temperature. It was found necessary to have three coils in the inter-



Wiring connections between thermostat, relay and heating unit.

mittent circuit to take care of large declines in laboratory room temperature caused by interruptions of the general heating. If these could be avoided, one coil would suffice for the intermittent circuit in a smaller incubator than those in use here. One electric lead between heating unit and instrument panel is common to both heating circuits for simplicity. The heating unit is placed on the floor of the culture chamber. It could be placed immediately beneath and in contact with the bottom of an incubator, provided with a water jacket, in which case, however, a larger heating capacity of the unit would be required. The three insulated leads from the heating unit to the binding posts in the panel pass through the same ventilating port as the capillary tubing of the thermostat. In the incubator room, an electric stove serves as the constant heat source and an electric broiler of household type as the intermittent heat supply.

To maintain uniform temperature throughout an incubator requires adequate ventilation: the ventilating ports must be of sufficient size, must be open and not entirely obstructed by mercury thermometers passed through them. The constancy of temperature within an individual incubator depends largely on the length of time the doors are open, and is greater in an incubator with a water-jacket than in one not so supplied with a heat reserve. With the doors constantly closed, temperature changes within the incubator occur so slowly that they may be followed precisely by a recording thermometer.[6] With room temperature between 18 and 20 C., the variation at a given level within the incubator has been too small to read, or less than 0.5 C.,[7] and in a chamber 24 inches in height the variations between the levels from 3 inches above the bottom to 5 inches from the top have on each occasion tested been about 1 C. over a forty-eight hour period. In consequence the recording thermometer has been used mainly to check new installations.

The diagram gives the wiring connections between the different parts of the apparatus. Soldered electric connections are unnecessary.

THE STABILITY OF KOLMER'S ANTIGEN FOR COMPLEMENT FIXATION TESTS IN SYPHILIS

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In a previous communication,[1] it was reported that antigen extracts prepared according to the method described by Kolmer[2] could be kept, without special precautions, for a period of twenty-one months without any evidence of deterioration other than a slight increase in the anticomplementary unit.

Although intended primarily for use in the method of complement fixation described by Kolmer,[3] because of its low hemolytic and anti-complementary properties and the high antigen unit common to these preparations, this antigen has been utilized in a variety of methods.

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6. On the vapor tension principle, giving temperatures from 10 to 110 C. on a twenty-four hour chart. Manufactured by the Brown Instrument Co., Philadelphia.

7. Control by instruments of the type described here is not independent of the barometric pressure. At constant temperature the sensitive arm must undergo movement with variations in barometric reading. Such changes are so small as to be of no practical significance; the maximum change with 1 cm. of mercury change in barometric pressure is 0.08 C.

1. Kilduffe, A. A.: The Stability of Kolmer's New Antigen for Complement Fixation Tests in Syphilis, J. Lab. & Clin. Med. **9**:781 (Aug.) 1924.

2. Kolmer, J. A.: Studies in Standardization of the Wassermann Reaction: A Superior Antigen for Complement Fixation Tests in Syphilis (A Cholesterolized and Lecithinized Alcoholic Extract of Heart Muscle, Am. J. Syph. **6**:74 (Jan.) 1922.

3. Kolmer, J. A.: Studies in the Standardization of the Wassermann Reaction: A New Complement Fixation Test for Syphilis Based Upon the Results of Studies in the Standardization of Technic, Am. J. Syph. **6**:82 (Jan.) 1922.

Probably no single reagent required for the conduct of the complement-fixation test in syphilis is of greater importance or exercises a more profound effect on the sensitivity and reliability of the reaction than the antigen extract; any information, therefore, concerned with or tending toward uniformity in the preparation of antigen extracts is of interest and practical importance.

It has been shown previously[4] that beef heart muscle powder can be prepared commercially in large quantities representing a large number of hearts, and thus possessing a high degree of polytropicity; that such powders can be kept under ordinary conditions and without special precautions for as long as one year without deterioration; and that these preparations were, without exception, suitable for the preparation of highly antigenic Kolmer antigens.

The practical application of these observations is obvious in that, regardless of the nature of the antigen extract desired, a uniform and stable source of heart muscle powder is available for its preparation.

As the preparation of antigen extracts involves a definite degree of expense, and as certain smaller laboratories are accustomed to purchase this reagent; and as, under certain circumstances, its distribution to branch or subsidiary laboratories from a central source of supply comes under consideration, it becomes of interest to ascertain whether such extracts are sufficiently stable to permit their preparation and storage in bulk.

The present communication is concerned with a further study of the stability of antigen extracts kept under ordinary conditions and without special precautions for varying periods.

All antigens were titrated in exact accordance with the method described by Kolmer, using an antisheep hemolytic system.

Antigen P^1, when prepared on April 6, 1922, had the following titer: hemolytic: not in 0.5 cc. of 1:4; anticomplementary: 0.5 cc. of 1:4; antigenic: 0.5 cc. of 1:2,400. This extract, after storage in the ice chest was shipped by freight to Los Angeles, stored, shipped by freight to Trenton, N. J., stored in a warehouse two months, and shipped by freight to Atlantic City.

When titrated again after forty-eight months, the anticomplementary unit was 0.5 cc. of 1:10 and the antigenic unit 0.5 cc. of 1:2,400.

Antigen P^2, under the same circumstances, showed an increase in the anti-complementary unit of from 1:4 to 1:12 and a decrease in the antigenic unit from 1:2,400 to 1:2,000.

Several extracts (series E), prepared in Los Angeles, shipped by freight to Trenton, stored in a warehouse and again shipped by freight to Atlantic City, were again titrated when 28, 26 and 30 months old; all presented comparable changes, the anticomplementary units showing a slight increase and the antigenic units remaining unchanged.

While the results of antigen titrations are obviously influenced by varying degrees of complement activity, variations in cell suspensions, and the like, the results reported of the study of eleven antigen extracts indicate that extracts prepared in accordance with Kolmer's method are relatively stable and withstand extremes of temperature and long storage with relatively little change in titer.

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4. Kilduffe, R. A.: The Stability of Desiccated Beef Heart Muscle Powder for Preparation of Antigens for Conduct of Complement Fixation Tests in Syphilis with Special Reference to the Kolmer Method, Arch. Dermat. & Syph. **10**:734 (Dec.) 1924.

General Review

EXPERIMENTAL TAR CANCER*

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Industrial Tar Cancer

Experimental Tar Cancer
- Historical Introduction
- Irritants Other than Tar
- The Susceptibility of Various Species
- The Chemistry of Tar
- The Technic of Applying Tar
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  - Size of the tarred area
  - Interval between tarrings
  - Cessation of tarring
  - The latent period
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  - The percentage of success
- The Constitutional Effects of Tarring
- The Local Effects of Tarring
  - Macroscopic lesions
  - Microscopic lesions { Epithelium / Connective tissue
- The Tar Tumor
  - Inception of malignancy
  - Carcinoma
  - Sarcoma
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- Etiology
  - Local or Constitutional Action of Tar in Etiology
    - Soil
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    - Castration or splenectomy
    - Insulin
    - Trauma

*From the Institute of Cancer Research, Columbia University, F. C. Wood, Director.

INDUSTRIAL TAR CANCER

It has been known for years that those whose occupations expose them to soot, tar, or similar substances are prone to develop cancer, and the conditions governing its occurrence have been to some extent delimited.

Thus Ross [229] has observed that blast furnace tar, though similar to coal tar, except that it is distilled at a lower temperature and from a different variety of coal, appears to be harmless, whereas gasworks tar causes warts and epitheliomas on the hands and arms of the workmen who handle it; the incidence is not so high, however, as in the case of pitch or soot.

Differences in the nature of the coal employed have been advanced by Courmont [58] to explain the frequency of industrial tar cancer in England and its extreme rarity in France.

A prolonged exposure to the irritant is usually necessary, ten years having been the shortest period in the experience of O'Donovan,[202] and forty years the longest. In one case the patient had not handled tar for twenty-eight years, and this writer cites an instance recorded by Bland Sutton in which soot cancer developed thirty-five years after the cessation of exposure.

Age did not seem to be a factor of importance in O'Donovan's series. In sixteen patients, the carcinoma developed at: 33 to 39 years in 4 cases, 40 to 49 years in 1 case, 50 to 59 years in 3 cases, 60 to 69 years in 6 cases and 70 to 75 years in 2 cases.

O'Donovan regards the prognosis as good, and says that papillary tumors that have microscopically been proved carcinomatous have generally fallen away after from two to four months' duration.

In this his experience has been similar to that of others. Schamberg,[248] for example, regards as a notable feature of tar cancers their

frequent tendency to undergo spontaneous involution, and says that older writers have remarked on the benign nature of chimney sweeps' cancer of the scrotum.

The almost universal restriction of this epithelioma to the scrotum has been the subject of considerable discussion since the disease first came under observation, but Kennaway [135] has recently called attention to the fact that other occupational cancers also have a definite site of election. Two thirds of all pitch cancers occur about the face or scrotum, other parts, equally exposed to pitch dust, seldom being affected. In mule-spinners' cancer the scrotum is again the site of election, although the penis and a wide area of skin on the abdomen and thighs are equally exposed to the oil which causes this neoplasm. In aniline dye workers, cancer develops almost always in the bladder, and on its posterior wall; while in persons taking arsenic, it is the fingers, legs and trunk that are most often attacked. In coal miners and agricultural laborers, carcinoma involves the penis much more frequently than the scrotum; therefore, says Kennaway, neither the rugosity of the scrotum nor the abundance of its sebaceous glands can explain the frequency of cancer at this site in certain trades.

The carcinogenic activity of mineral oils, which cannot be gone into here, has been discussed by Scott [246a] and Leitch.[155a]

Although years of exposure are usually necessary for the development of occupational cancer, as has already been indicated, this seems not invariably to be true. Thus Huguenin [105] has described the case of a workman, aged 35, who had often suffered burns from droplets of crude oil, which had left no trace. In the present instance, however, the hot oil fell on the recent scar of a deep burn received from a charcoal-stove, where it caused a burn of the first degree. One week later a small papule appeared, which grew so rapidly that it had attained the size of a walnut within twenty-five days. Histologic examination of the extirpated nodule showed that it was a keratinizing carcinoma.

Bang [5] has reported a somewhat similar instance in a gas retort workman, in whom cancer of the nostril developed sixteen days after a tar burn.

The preceding cases have been cited, not with the idea of giving even a fragmentary review of industrial cancer, but because there is not one but what can be matched in the laboratory. The similarity between industrial tar cancer in man and the experimental tar cancer of certain laboratory animals is close indeed.

EXPERIMENTAL TAR CANCER

HISTORICAL INTRODUCTION

The existence of occupational cancer could hardly pass unnoticed by any pathologist interested in the genesis of malignant tumors, and the

disease has, in fact, inspired a long series of experiments. Nearly forty years ago, Hanau [101] painted rats for months with gas tar or similar materials, but elicited only a chronic dermatitis. At the close of his paper he suggested that a long period of application might prove successful. It would almost inevitably have failed in rats, as we now know; but if he had only happened to choose the mouse for his investigation, instead of the rat, there is no doubt that he would have triumphed.

Cazin,[54] a few years later, made an equally unfortunate choice—the dog; and in spite of five months' tarring, no carcinomas were produced.

Nearly twenty-five years ago, Bashford,[10] in drawing up a scheme for cancer research, suggested that "systematic study of the effects of persistent irritation of different epithelial surfaces in different species of animals might be found to have important bearings," and went on to suggest a study of petroleum cancer and sweeps' cancer. But this was just at the time when the attention of every one was suddenly turned toward Jensen's work with transplanted tumors, and the proposal was not carried out.

Tobacco tar was applied to the ear in rabbits by Wacker and Schmincke,[266] and soot to the ear or scrotum of the same species by Haga.[99] This time the right species of animal was chosen, but as fate would have it, the irritant was applied for too short a period to instigate malignant growth.

The most promising of the older experiments was that of Bayon,[14] who reported abundant epithelial proliferation, but without invasion, four weeks after the injection of gas-works tar into the ear of a rabbit. Once again the soil and the irritant were right, and only sufficient time was wanting. Blast furnace tar, on the contrary, excited practically no response from the epithelium.

Thus in Germany, in France, and in England the problem of inciting malignant growth at will has been on the eve of solution for the past forty years, but the experiments failed either because an insusceptible animal was unwittingly chosen, or because irritation was not continued for a sufficiently long period of time. It was the good fortune of two Japanese investigators to select an animal sensitive to the irritant which they employed, and to be possessed of the infinite patience necessary for applying tar week after week, month after month, without any prospect that their labor would be rewarded.

In 1914, Yamagiwa and Itchikawa [272] reported that they had been able to produce papillomas on the ear of the rabbit by repeated applications of tar, and in the following year, they [273] recorded the development of three cancroids in the tarred animals. The wide confirmation which was their due was delayed, however, by the World War, and it was not until the conclusion of hostilities that it came.

IRRITANTS OTHER THAN TAR

In the course of their preliminary experiments, Yamagiwa and Itchikawa tried various mechanical and chemical irritants,[280] but found tar to be by far the most active. Burckhardt and Müller[44] say that repeated burning, scalding or exposure to the roentgen ray were all negative, whereas tar was promptly successful; and eosin, hematoxylin, gentian violet, picric acid, silver nitrate, phosphorus, arsenic, ichthyol and balsam of Peru, all proved inoperative in the experiments of Daels.[61] Yet Leitch[154] succeeded in one mouse with arsenic, although he found tobacco extract, benzene, benzidene, xylol, aniline, various dyes, iodine, zinc chloride, products of intestinal putrefaction, cresols and numerous tar products inactive. He accordingly suggested that the various carcinogenic agents are specific and selective, and that the development of carcinoma is not due to mere irritation.

Teutschlaender,[256] on the other hand, believes that the action of tar is not specific, since this agent will produce inflammation, benign growths or malignant growths. He does not agree with Lubarsch,[168] however, that no importance is to be attached to the agent itself and all importance to the intensity of its action, for, as he points out, certain parasites produce cancer while others do not; arsenic is carcinogenic, whereas other elements are inoperative. Thus tar may be said to have a relative specificity.

Derom[77] was unable to excite malignant growth with tincture of iodine or with dilute phenol, and Rosenstirn[228b] failed with iodine and chrysarobin, but Narat[200] succeeded in eliciting carcinoma in two strains of mice by painting with 3 to 6 per cent potassium hydroxide or 3 to 5 per cent hydrochloric acid. The lesions were essentially similar to those caused in the same strain of mice by crude tar, although tar gave a higher percentage of tumors. Pachydermia developed after about ten weeks of painting, and papillomas in from four to five months. In about 15 per cent of the animals painted with caustic potash or hydrochloric acid, the lesions progressed after the irritant had been discontinued, gradually increasing in size and invading fat, muscle and the peritoneal cavity. No metastases were discovered, and both autotransplantation and homotransplantation failed; but the tumors recurred after wide excision in six of nine animals operated on.

In two other strains of mice, potassium hydroxide and hydrochloric acid produced only benign growths, and in these mice tar was also inactive.

THE SUSCEPTIBILITY OF VARIOUS SPECIES

Tar has been applied to all the common laboratory animals, none of which, however, has proved so susceptible as the rabbit and the mouse. According to Yamagiwa,[271] tar painting will not produce carcinoma in

rats or fowls. Paszkiewicz [211, 212] painted white rats every two or three days or gave them subcutaneous injections every eight to ten months, but none developed a tumor.

A tar which elicited cancer in a rabbit after 136 days was found by Menetrier and Surmont [181] to be quite inert in the guinea-pig, although only a small number were tarred.

Leitch [151, 153, 155] also failed with guinea-pigs after two and one-half years of tarring; rats proved equally insusceptible, although tar produced the same skin changes in them that it does in mice, and not even a papilloma appeared in several series of rats painted for more than a year.

Polettini [217] applied tar to rats in vain for six months, and Mertens [184] was just as unsuccessful with guinea-pigs to which tar, anthracene oil or asphalt were applied.

According to Itchikawa,[109] rats and dogs are insusceptible, and Itchikawa and Baum [115] saw no tumors in either rats or guinea-pigs, although the former remained alive for three months and the latter for six.

The guinea-pig was found insusceptible to tar painting, also, by Dentici,[75] Halberstaedter,[100] Teutschlaender,[256, 257] Borrel, Boez, and de Coulon,[39] and Bloch.[30]

According to Teutschlaender,[256] neither pigeons nor hens react to tarring, and Borrel, Boez, and de Coulon [39] agree so far as fowls are concerned.

In the experiments of Choldin,[55a] simple papillomas were produced in two chickens only, whereas all tarred mice developed tumors. He therefore regards species predisposition as of much greater import than individual predisposition for carcinogenesis.

Buschke and Langer,[48] Borrel, Boez, and de Coulon,[39] Halberstaedter,[100] Deelman [68] (who suggests the thick layer of keratinized epithelium in this animal as a possible reason), Teutschlaender,[256] and P. Möller [190] have all failed with rats. Dentici [75] records the appearance of a simple papilloma in one rat out of thirty, after 176 days' painting.

The only investigator who has produced a carcinoma in the skin of the rat is Herly,[103] whose experiments were carried out at the Crocker Institute. One hundred white rats were painted for 226 days with a gas-works tar known to produce carcinoma in mice, which was diluted to 50 per cent with glycerine; to this mixture, 1 per cent of arsenious acid was added. One hundred control rats were painted with 1 per cent arsenious acid in glycerine.

On the 226th day, alopecia, pachydermia and a few papules were observed in the tarred animals, but the controls exhibited no change.

For reasons not connected with the experiment, painting was suspended at this point for five months. At the end of this period sixteen

of the tarred rats were still living, and applications were resumed on the three hundred and eighty-first day after the beginning of the experiment, and continued until the four hundred and forty-fourth day. They were again interrupted, this time for two months. On the 511th day, one of the survivors had a cutaneous horn with a thickened and infiltrating base, similar in all respects to those of mice. Portions of the base were removed for transplantation and microscopic examination. The death of the rat prevented autotransplantation, but the microscope showed the tumor to be a carcinoma which was invading the underlying musculature. Transplantation into fifty-five other rats was undertaken. In three the graft grew temporarily, reaching the size of a pea in from four to nine weeks. In one rat the graft grew progressively, though slowly, and when the rat died, six and one-half months after inoculation, was about the size of a hickory nut.

Stefko [253] reported the development of lymphangiomas in frogs and toads, but no carcinomas; still, these amphibia were found to be so susceptible to tar poisoning that they died after three or four weeks of painting.

In order to throw some light on the manner in which tar incites malignant growth, Reiss [220, 221] tried its effects on the sea urchin's egg. Coal-tar or wood-tar was shaken up in sea water, and a few drops of the extract thus prepared were added to 50 cc. of sea water containing the eggs. Nonfertile eggs were untouched, even by stronger preparations, neither their division nor their permeability being affected, but fertile ones exhibited abnormal division figures in the solution described. Ether and petroleum extracts, however, were inert; but substances other than tar produced mitotic anomalies similar to those called forth by the watery preparation of tar.

The nuclear changes included unequal chromatin distribution and depolarization of mitoses, and as similar alterations are found in cancer, Reiss suggested that this disease may be due to some affection of the nucleus.

Although the skin of rats, guinea-pigs and fowls is almost totally resistant to the carcinogenic action of tar, other of their tissues are not. Maisin and Picard,[175] recalling Fibiger's observation that the stomach and tongue of rats respond to the presence of *Spiroptera neoplastica* by producing carcinoma while the esophagus is entirely refractory, instituted a series of experiments to determine whether the mucous and serous surfaces in the rat share the exemption of the skin. Small blocks composed of a mixture of equal part of paraffin, tar, and scharlach R were placed in the urinary bladder, pleural cavity or peritoneum, and one of the rats in which the irritant was placed in the bladder received additionally an injection of one drop of tar into this organ thirty-four days

after insertion of the block. When the animal died, one hundred and fifteen days after the beginning of the experiment, a papillary carcinoma was discovered on the posterior wall of the bladder, which had invaded the muscular coat and metastasized in a lymph-node.

Menetrier [178] injected 1 or 2 drops of tar into the perigastric, perihepatic and perirenal regions of a rat, twelve such injections being given in 349 days. Oil of cade was subsequently introduced, which caused an abscess, and one injection of creosote was given, which led to the death of the animal on the 341st day. In the cardiac end of the stomach, where the epithelial coat is squamous, a carcinoma was found. The tar had not penetrated the stomach wall, but as the tumor lay exactly beneath a mass of it outside the stomach, Menetrier assumed that the neoplasm had been caused by some soluble material able to pass through this viscus and influence its epithelium. In other experiments, he made injections into the pancreas and the testis, but in neither of these organs was any neoplastic proliferation induced.

A detailed account of this tumor will be found in a subsequent paper by Menetrier and Derville,[180] in which it is described as a papillary carcinoma, infiltrating the muscular wall.

Teutschlaender [258, 258a] introduced tar into the vaginas of seven rats, one of which developed a carcinoma of the uterus that penetrated its wall. The tumor was discovered in that region of the uterus in which tar is most often found after intravaginal injection, 242 days after the first injection and 173 days after the last.

Two tar sarcomas have been described by Russell [240, 241] in four survivors of forty rats that received weekly a subcutaneous injection of from 10 to 15 mg. of tar for one year. The first appeared eleven and one-half months after the beginning of the experiment, and increased rapidly in size. After two weeks it was extirpated and transplanted into the rat itself and into other rats. Both autoplasts and homoplasts grew vigorously; the primary tumor recurred, and at necropsy two pulmonary metastases were found. Microscopic examination showed the tumor to be a polymorphous cell sarcoma.

The second sarcoma appeared eighteen months after the beginning of the experiment, or six months after injections of tar had been discontinued, and grew with fair rapidity. It was removed and transplanted into the animal itself and into other rats; two weeks later, at necropsy, the autoplast showed no sign of growth and no metastases were found, although the grafts proliferated well in the other rats. The tumor was a polymorphous cell sarcoma containing spicules of bone.

Bone was seen in only two daughter tumors, which were ninety-three and ninety-eight days old, respectively. Thus a considerable length of time appeared to be necessary for the formation of bone, and its absence in most of the daughter tumors was ascribed by Russell to their rapid

growth, which destroyed the host long before the requisite three months had elapsed.

He regarded this second tumor as particularly interesting because of the lengthy period (nearly six months) elapsing between cessation of tarring and the detection of a growth. The interval was, in fact, much longer than any observed in mice by Leitch, who had made a special study of this point. Russell thought it difficult to decide, however, how much of the interval was real. If one were to judge solely by the size of the tumor when first discovered, and by its subsequent rate of growth, the date of onset would be put back only three or four weeks; but if the degree of osteoid metaplasia were compared with that shown by daughter tumors of known age, the date of onset would have to be set back four or five months, and the period between the cessation of tar injections and the inception of neoplastic change would be practically wiped out. Thus this case could not safely be regarded as exemplifying the onset of neoplasia long after the exciting cause had ceased to operate.

In the guinea-pig, Kazama [128, 130] has reported the presence of adenocarcinoma of the gallbladder following the injection of tar into its cavity. Although this work in itself is not quite so convincing as might be wished, it gains considerable strength from its confirmation by Leitch,[155] who has proved that it is comparatively easy to produce carcinoma in the gallbladder of the guinea-pig. Leitch is extremely critical of his own results, and permits himself the diagnosis of carcinoma only on demonstration that the growth has invaded adjacent structures, such as the ribs, liver or omentum. Of these instances no less than eight had already been encountered among the survivors of twenty-five guinea-pigs that had had pebbles, gallstones, or pilules of pitch introduced into the gallbladder from five and one-half to twelve months previously, and some of the animals were still living when the paper was written.

Polettini [217] has given an account of the production of an adenoma destruens in the gallbladder of the guinea-pig, by injection of tar into the cavity. Invasion of the muscular coat had begun.

Although no carcinoma has yet been brought about in the fowl, Carrel [49] and Murphy and Landsteiner [192] have reported tar sarcomas. Carrel describes his as the most malignant of all known tumors, since it may kill its host in four or five days. Murphy and Landsteiner injected into ten adult hens 0.5 cc. of a mixture of equal parts of minced chicken embryo and tar extract; every two weeks thereafter, 0.2 cc. of tar extract was introduced into the resulting mass in the breast. After five months, three of the hens were still living, and two of these had progressively growing tumors at the site of injection, which later microscopic examination showed to be spindle cell sarcomas. In one of the hens, which died during the fifth month, no metastases were found, and although her tumor was transplanted into fifty-six chickens, no progres-

sively growing tumors developed. The second one died during the seventh month, with metastases in the liver and lungs. In one of fifty chickens inoculated with her tumor a sarcoma resulted, which had been transplanted for eleven generations when the paper was written.

In man himself, two epitheliomas have been recorded as a result of tar painting. De Jong, Meyer and Martineau [72] observed the development of a squamous cell carcinoma in a man 85 years old, after eight years' constant painting of an eczematous area with coal-tar ("goudroline"). This the patient himself applied, against the advice of his physician, because it was the only thing that would allay the intolerable itching. As eczema, according to the authors, is not known to be followed by cancer, they believed it reasonable to implicate the tar.

The second instance was that of a 68 year old man reported by Veiel,[264] who had suffered from recurrent eczema of the scrotum for twenty-three years. He had frequently applied a 33.3 per cent alcoholic solution of pine tar, thus producing an inflammatory lesion on which papules appeared, one of which became malignant. Like De Jong and his associates, Veiel ascribed the carcinoma to the tar rather than to the eczema, since the manner in which it developed exactly resembled the origin of tar carcinoma in animals.

In this connection it is important to note that Sternberg [254] has produced cancer in three mice with three tar preparations that are used in the treatment of psoriasis—carboneol (a solution of tar in carbon tetrachloride), carboterpin (a solution of tar in terpinol), and lithantrol (an alcoholic preparation). In two of the mice there were pulmonary metastases.

There may be mentioned in conclusion the suggestion of Vaughn [263] that, as kangri cancer and the skin surrounding it are begrimed with a material resembling tar or soot and resisting soap and water, this variety of carcinoma may be a tar cancer rather than one produced by heat.

Experiments with various species of animals such as those which have just been described indicate that the production of a malignant tumor is not the result of mere chronic irritation, but that there must be a specific relation of some sort between tissue and irritant. And, indeed, as will appear in subsequent paragraphs, it has even been proposed that not every cell in a given tissue is susceptible to the carcinogenic principle, but that the specific relation may exist only between certain cells and the active agent.

THE CHEMISTRY OF TAR

Obviously, it is important to know what constituent in tar is responsible for the initiation of carcinoma. Not that any one expects to find a specific substance possessed of unique carcinogenic activity, for the work of the past few decades has clearly shown that there are several, if

not many, agents which are able to originate malignant growth. But it might become possible to produce experimental cancer in a shorter time; or to lower the excessive death rate of experimental animals from tar poisoning; or to study the earliest stages of carcinoma unembarrassed by the concomitant chronic inflammation set up by tar; or, most important of all, to eliminate tar cancer as an occupational disease, if the active principle could be isolated.

The problem, however, is one of extreme difficulty, for several hundred substances have been identified in coal-tar, though not more than one hundred have been definitely isolated.[252] Then, too, the composition of tar varies considerably, depending on the coal from which it is distilled. According to Lunge,[169] the tar obtained from (Scottish) blast furnaces worked with splint coal is entirely different from gas-tar, containing very little aromatic hydrocarbons and phenols of a quite different character.

Most of the constituents of coal-tar, which is also called gas-tar, belong to the aromatic hydrocarbons, from benzene, the simplest and most volatile, up to a mass of indistinguishable, nonvolatile bodies that compose the pitch which remains in the stills. These aromatic hydrocarbons are chiefly of the benzene, naphthalene, anthracene and phenanthrene series. Small quantities of aliphatic hydrocarbons, also, belonging mainly to the paraffins and olefins, are regularly present. Besides its hydrocarbons, coal-tar contains phenols, sulphur compounds, and nitrogenated compounds which are mostly of a basic character and belong to the pyridine and quinoline series.[169]

The number of fractions taken during distillation varies from four to six. Sometimes a fraction is taken as "first runnings" or "fore runnings," up to a temperature of 105 C. in the still, and a second fraction, as "light oil," up to 210 C.; but more usually these two are taken together, when they constitute the light oil fraction. The next fraction, "middle oil," or "carbolic oil," coming off up to 240 C., contains most of the phenol and naphthalene, while the third is the "heavy oil," or "creosote oil," made at 270 C. Above this point "anthracene oil," or "green oil," is obtained up to the end of the distillation; the point at which distillation is terminated depends on whether a soft or a hard pitch is desired. The temperatures at which the various fractions are taken off vary somewhat, however, at different works, and these figures are reproduced from Lunge's article [169] in order that the reader may compare them with others to be given in subsequent paragraphs.

One of the earliest papers to discuss the carcinogenic agent in coal-tar was that of Bloch and Dreifuss,[31, 80] who said that it is contained in a fraction with a high boiling point (370 to 440 C.), which is soluble in benzol and contains none of the hydrocarbons, bases, or phenols of a low boiling point. This fraction caused extremely malignant tumors in 100

per cent of mice in about four months. Shortly afterward, Bloch [28] reported that nitrogen-free fractions are active, a statement which may be compared with Philippson's assertion [216] that the agent concerned in industrial cancer is one of the nitrogenous organic bases.

It was next found by Deelman [63, 66, 68] that tar from horizontal retorts evokes cancer in mice in a shorter period than that from vertical retorts, though both cause the same kind of lesion.

It is generally agreed that the carcinogenic agent is present in the distillates that come over above 200 C. Thus Jordan [122] says that the precancerous stage was obtained with a residue left after distillation at 400 C. in about half the time required by whole tar, but that the experiments had not been in progress long enough for cancer to develop. The distillates, including anthracene oil, were either inactive or so poisonous that the mice died within a very short time, and in some cases immediately. Teutschlaender [256] and Kennaway,[132] however, both succeeded in producing cancer in mice with anthracene oil.

Jordan gives the following fractions and the temperatures at which they come over:

1. First distillate (up to 70 C.): Volatile hydrocarbons, ammonia, etc.
2. Light oil (up to 180 C.): Benzol, bases, aromatic hydrocarbons paraffin, etc.
3. Middle oil (up to 240 C.): Phenol and naphthalene.
4. Heavy oil (up to 300 C.): Neutral and acid oils, naphthalene, etc.
5. Anthracene oil (over 300 C.): Crude anthracene.
6. At about 400 C. there remains a residue of pitch.

It will be noted that these temperatures vary a little from those given by Lunge, which is to be expected since the practice varies at different works, as has already been said.

Hoffmann, Schreuss, and Zurhelle [104] tried seven fractions, as pure as they could be made, but succeeded only with a neutral oil coming over at 200-380 C. There seemed to be a difference in the type of tumor produced by various materials, for mice painted with this neutral oil developed hemispherical papules, whereas whole tar gave rise to ulcerating papillomas, but the authors were not inclined to attribute too much significance to their few preliminary experiments.

Mertens [185] found that an oil distilled at 280 to 380 C., and thus freed of most of its phenols and bases, produced carcinoma in neither mice nor rabbits, while Petit [215] produced tumors in less than four months in mice and rabbits with a product coming over above 300 C. and rich in acridine ($C_{13}H_9N$).

De Coulon [62] points out that while gas-works tar is a mixture of substances belonging to the aromatic series, vacuum tar is a mixture of hydro-aromatic bodies analogous to petroleum. In its preparation, distil-

lation is stopped at 450 C., the temperature at which distillation is beginning in the case of gas-tar. It appears to lack the carcinogenic factor.

Maisin, Romme, and Jacqmin [175c] also said that tar distilled at about 450 C. was noncarcinogenic, as were the constituents coming over below 200 C., at 200 to 300, and at 300 C. Even a benzol solution of the pitch was inactive. In spite of numerous attempts these authors failed to produce cancer with chemically pure coal-tar constituents, such as anthracene, phenanthrene, carbozole and acridine.

According to Fukuda and Kinoshita,[90] the volatile oils obtained during the distillation of tar are noncarcinogenic. Yet Bierich [16] produced cancer, though only in a single mouse, with pyrrole, a substance with a low boiling point (133 C.).

As the carcinogenic agent is found in the fractions with a high boiling point, Deelman [68] assumed that it must be present in pitch, and endeavored to dissolve it out. He found that it is soluble in toluol, benzol, or acetone, and that the remaining pitch is then inactive. When a toluol solution of pitch is distilled, the fraction which comes over at 150 to 255 C. is more active than those coming over at from 255 to above 275 C.

Teutschlaender [256, 257] also succeeded with a benzol solution of pitch, although it did not act any earlier or with any more certainty than whole tar.

Maisin [171] found, contrary to the early work of Bloch and Dreifuss, that substances of relatively low boiling point (300 to 350 C.) may be carcinogenic for mice, as cancers of rapid development and great malignancy could be produced in three or four months, although the most active fraction was that boiling above 350 C. A benzene extract of pitch obtained by evaporating at 350 C. a sample of coal-tar from a horizontal retort was particularly active, and not so toxic as the lower fractions.

Jordan [122] painted twenty mice, five of which lived more than 100 days, with a solution of whole tar in xylol. On the 112th day, one of the five had a small papilloma which microscopic examination showed to be nonmalignant.

Parodi [206] reported that the active principle cannot be removed with ether, xylol or benzol. His experience, however, does not coincide with that of others; for, as Maisin [171] says, every one agrees that the carcinogenic principle can be removed with benzene; and benzol is commercial benzene, containing toluene and small amounts of xylene and other substances.

In contrast with Parodi, Murray [196] prepared an extremely active sample by extracting coal-tar successively with water, alcohol and ether. The ethereal extract gave 50 per cent of malignant growths in twelve months, reckoned on the total number of animals surviving for four

months. In an experiment in which whole tar, alcoholic extract, and ethereal extract were applied to separate areas of the dorsal skin in sixty mice, fifty of the animals survived for four months or more, and twenty-five of them bore malignant tumors. Twenty-two of the twenty-five had carcinoma at the site painted with ethereal extract, twelve at that painted with whole tar, and only two as a consequence of applying alcoholic extract. Murray believed, however, that the experiment did not truly represent the potency of the alcoholic extract.

No doubt the conflicting results reported by various authors are due in part to differences in the tar employed, for, according to Bloch,[30] there are tars in which the carcinogenic agent is totally lacking; hence none of the well-known constituents common to all tars can be the active principle. Furthermore, he suggests that there may be more than one carcinogenic factor.

Schreuss and Zurhelle[245] have found wood-tar as efficacious as coal tar.

By far the most extensive and valuable investigations into the relations between the chemistry of tar and its carcinogenic power are those of Kennaway.[131] He gives the following list of the principal forms of tar:

Coal-tar
1. Wood-tar
2. Lignite-tar
3. Gas-works tar
   (*a*) Horizontal retort
   (*b*) Vertical retort
4. Blast-furnace tar
5. Coke-oven tar
6. Producer-gas tar
7. Water-gas tar

Of these, lignite-tar, gas-works tar, producer-gas tar and probably coke-oven tar are known to be carcinogenic, while blast-furnace tar is not. There seems to be no information in respect to water-gas tar.

The table immediately below shows the differences between horizontal and vertical retort tar, as set forth by Kennaway.

Substances more abundant in	
Vertical	Horizontal
Low temperature (below 550 C.)	High temperature (900 to 1100 C. or more)
Paraffins	Free carbon
Olefines	Phenol
Naphthenes	Aromatic compounds other than higher phenols and methyl naphthalenes
Phenols other than phenol	
Methyl naphthalenes	

In blast-furnace tar the phenols as a class are much more abundant, and phenol proper (carbolic acid) much less abundant, than in gas-works tar. In this, as well as in the presence of higher paraffins in distinct quantities and the small amount or absence of benzene, naphthalene and anthracene, it resembles a low temperature tar.

At present it is unknown [132] whether the inability of blast-furnace tar to produce cancer either in men (Legge [149]) or in mice (Leitch [154]) is due to the nature of the coal from which it is distilled, the temperature of carbonization, or to some reaction dependent on the chemical conditions peculiar to a blast-furnace.

As attempts to find the carcinogenic agent among the known constituents of coal-tar have given wholly negative results, Kennaway suggests that this factor may be some unknown compound present in infinitesimal quantities. His paper gives an account of the various fractions of gas-works tar, and a bibliography, chiefly of industrial cancer.

In a second communication, Kennaway [132] combined a study of industrial and experimental tar cancer, and the following table is a combination of two of his with certain material in the text of his article. The figures in parenthesis represent the upper limit of temperature in the still at which each fraction is given off, while derivatives which are known to be carcinogenic are printed in italics. Kennaway points out, however, that the figures for temperature are only approximate, and vary somewhat in different tar-works.

Tar

- Ammoniacal liquor, Crude naphtha (180 C.)
- Light oil (225 C.)
- Middle or carbolic oil (275 C.)
- *Creosote oil* (275 C.)
- *Anthracene oil* (320 C.)
  - *40 per cent anthracene*: 85 per cent anthracene; Anthracene residue
  - *Green, strained, or dead oil*
- *Pitch*: *Pitch distillate* (550 C.); Pitch coke

The evidence for carcinogenic activity is partly industrial and partly chemical. In the case of fractions of low boiling point (naphtha, light oil, carbolic oil) the industrial evidence is almost wholly negative, nor was Kennaway able to produce cancer with them in the laboratory.

As for creosote oil, no conclusive experiments have been made with this fraction alone, but he recalls the fact that Bloch and Dreifuss had negative results with "low boiling hydrocarbons," which he takes to mean some fraction boiling below 300 C.; this might include creosote oil. There is industrial evidence, however, that creosote oil produces carcinoma.

Anthracene oil and green oil have both caused cancer in those who work with them, and with both of them Kennaway has produced cancer in mice. They are, however, very toxic for these animals, and the number of mice surviving was too small to permit any comparison of their relative potency.

There is industrial proof that 40 per cent anthracene can incite carcinoma, and Kennaway produced a papilloma in one mouse out of 100 painted with an ethereal extract of it. A similar extract of anthracene residue elicited no tumors, although he does not regard the test as very satisfactory, as only eight mice out of 100 lived more than 160 days.

Pitch often causes malignant epithelial tumors in those who handle it, and Kennaway and others (Leitch,[154] Passey,[208] Passey and Woodhouse,[210] Passey and Carter-Braine[209]) have produced carcinoma in mice with this material. Pitch distillate, too, Kennaway found to be distinctly carcinogenic for mice.

Thus both the industrial and the experimental evidence show that the carcinogenic factor may distil over at from about 250 to above 500 C. Kennaway has examined the compounds of high boiling point in tars and mineral oils, but has been unable to discover this agent. Anthracene, phenanthrene, chrysene, picene, retene, truxene, acenaphthene, fluorene, acridine, carbazole, aniline, benzene, toluene, and xylene were all excluded by experiment; and either experimental or industrial evidence, or both, is against naphthalene, the acids, bases, and other nitrogenous compounds, paraffins, olefines and naphthenes. He explains that the known constituents of coal-tar have all been isolated because they are abundant, or stable, or capable of forming definite compounds; and suggests that the carcinogenic principle may be among the unknown compounds, which have remained unknown because they are unstable or present in minute quantities only.

Acridine, which has often been suggested as the cancer-producing factor in tar, probably because of its irritating effect on the skin and mucous membranes, was applied to 200 mice, a large proportion of which lived for nine months but without developing cancer. Kennaway therefore concludes that irritation alone does not produce cancer, and recalls the observation of Legge,[140] who found no carcinoma among 175 cases of chrome ulceration in dyers, tanners and bichromate manufacturers.

Kennaway[133] discovered that when isoprene, $CH_2 : C(CH_3) \cdot CH : CH_2$, is passed through a tube filled with hydrogen and heated to 820 C., a mixture of compounds, chiefly aromatic, is formed which produces cancer more rapidly, and in a larger proportion of mice, than many samples of coal-tar. The interest of this substance lies, of course, in its simplicity, only the elements C and H entering into its composition. Kennaway suggests, however, that other materials may be present as impurities, although they can exist only in infinitesimal amounts; hence

it is impossible to assert that the carcinogenic substance contains only C and H.

He found, furthermore, that coal-tar made at 600 C. would cause cancer in mice, whereas overheated tar (1,000 C. or more) was remarkably feeble in this respect; the effect of temperature on the carcinogenic principle thus appears to be by no means simple.

It is suggested that inorganic compounds may act as catalysts, and Kennaway recalls Zanetti's discovery [289] that iron will inhibit the production of tar from a certain petroleum fraction, proposing that this inhibiting action by iron may explain the absence of carcinogenic power from blast-furnace tar.

The deduction of certain writers that the carcinogenic agent must contain nitrogen and must be a base, Kennaway believes to be unaffected by his experiment with isoprene tar, as the ultimate etiologic factor may be a secondary product elaborated by damaged tissues.

In a later paper, Kennaway [134] reports that substances more simple even than isoprene can be employed as a source of carcinogenic compounds. Thus acetylene, HC≡CH, when heated to 700 C., yields a tar which is distinctly active. Another tar made from acetylene at 800 to 900 C. was inferior in carcinogenic properties, though not entirely inert.

This acetylene tar resembles isoprene tar in the simplicity of its composition, for elements other than C and H can be present in traces only. It therefore offers a material less complex than coal-tar, which contains O, N and S as well.

A Californian petroleum which Leitch [155a] had shown to be devoid of carcinogenic power produced cancer after having been heated to 880 C. in a current of hydrogen. The changes brought about by heating petroleum include the conversion of aliphatic into aromatic compounds. The experiment shows that the petroleum products most dangerous to workmen are the strongly heated distillation residues, and, in fact, two of the few fully recorded cases which Kennaway was able to find in the literature occurred in men exposed to such materials.

Isoprene tar made at from 700 to 720 C. and consisting chiefly of unsaturated aromatic compounds, was much inferior in carcinogenic power to an isoprene tar made at 820 C. and composed principally of saturated aromatic compounds.

Experiments with horizontal and vertical retort tars did not give, in Kennaway's hands, quite such conclusive results as were reported by Deelman.[63, 66, 68] But as different coals may yield tars varying considerably in their chemical composition, he believes it necessary that the horizontal and vertical retort tars be distilled from the same coal before any conclusions can be drawn in respect to their carcinogenic activity.

Kennaway had at his disposal three tars made from the same coal, at 450, 560 and 1250 C., the last of which would correspond most nearly to the ordinary gas-works tar. Application of these samples to mice indicated that the 450 C. tar was much less active than the other two, and the 1250 C. tar somewhat more effective than the 560 C. Thus the carcinogenic principle is given off in small amounts even below 450 and 560 C. and continues to increase, though at a much slower rate, from 560 to 1250 C.

Tar produced at 920 C. from yeast or from human skin also caused carcinoma in mice.

Kennaway concluded that the carcinogenic action of tar, whatever be its origin, cannot be ascribed simply to irritation, for all irritants do not produce cancer. Blast-furnace tar and certain petroleums, for example, do far more visible damage to mouse skin than acetylene tar or isoprene tar, yet their application is not followed by cancer; and chlorinated acetylene tar is much more irritating than the original material, although its carcinogenic power is considerably less. Other irritants which he has found to be negative are acridine, already mentioned, and the product obtained by combining ethylene with phenyl magnesium bromide in the presence of nickel chloride. It seems, therefore, that the irritant which causes cancer must be of a special kind, or must act on some particular element in the tissues.

Kennaway could discover no invariable relationship between the temperature at which various materials are formed, and their carcinogenic activity. For while with coal-tar, isoprene tar and skin tar the highest temperatures gave the more active products, in the case of acetylene the tar formed at a lower temperature was more effective. But on account of errors inherent in measurements of the temperature at which a product is distilled, and in comparisons of different series of mice, he desires to draw no general conclusions at present.

The idea cannot but suggest itself, he says, that acetylene may be a decomposition product common to coal, mineral oils, yeast, skin and isoprene, and that the carcinogenic agent may be formed from acetylene: but he makes the objection that if this were true acetylene tar should be more active than any other. It is not, however, and Kennaway closes his paper with the tentative hypothesis that the yield of carcinogenic principle may depend largely on catalytic reactions which cannot yet be controlled.

In 1919, Bayet and Slosse [12] reported that notable quantities of arsenic had been found in all samples of pitch which they had analyzed, and that arsenic was present also in the blood and hair of a large proportion of those who handled pitch. Bayet [13] therefore believes that tar cancer is more properly arsenic cancer, and has recently discussed at length [13a] the identity between the skin lesions of chronic arsenical

poisoning and chronic tar poisoning. He regards as additional proof the demonstration by Slosse of arsenic in the tissues and excreta of workers in tar and its products, and even in the tissues of tar painted animals, and suggests, accordingly, that in experiments on the production of tar cancer in animals account must be taken of the presence of arsenic in the tar.

As it had been known for years that the prolonged use of arsenic may be followed by keratosis and even epithelioma in the human subject, this seemed to be a promising clue, and Bayet's suggestion has therefore been carefully followed out. It has been shown by Leitch and Kennaway [157] that arsenic in the form of a 0.12 per cent alcoholic solution of potassium arsenite actually will produce cancer in the mouse when applied thrice a week, though they succeeded in only one mouse out of several series. After eighty-six days, a little papilloma appeared, which had reached a diameter of 1 cm. by the 140th day. On the 162nd day, when the mouse was killed, the carcinoma had infiltrated the muscle and metastasized to the lung.

Although arsenic is thus undoubtedly carcinogenic to some degree, other experiments do not suggest that it is responsible for tar cancer. Thus Leitch [154] showed that blast-furnace tar from Scotland would not incite carcinoma in mice, in spite of the fact that it contained as much arsenic as a sample of tar which induced tumors in five weeks; neither of the two tars contained more than a trace of arsenic.

Fibiger [83] and Fibiger and Bang [83, 89] produced metastasizing and transplantable tumors in mice with a tar that contained but 0.0003 per cent of arsenic, whence they concluded that arsenic is probably not the carcinogenic factor in tar.

A tar which in Jordan's experiments [122] caused cancer in every one of six mice that survived four months of painting, contained no arsenic, nor was it radioactive. The presence of metastases in five of these six mice leaves no doubt as to the diagnosis.

An equally effective tar employed by Teutschlaender [256] was also arsenic-free, although it evoked cancer in all mice that lived for four months or more.

De Coulon [62] worked with five samples of tar, painting 200 mice in five lots of forty each. He gives the following table, which contains the sources of the tars, the number of tumors produced by each one, and the arsenic content in mg. per hundred grams:

Paris	0.31 mg.	53 tumors
Strasbourg	0.40 mg.	40 tumors
Zurich	0.34 mg.	95 tumors
Lausanne	0.36 mg.	69 tumors
Geneva	0.80 mg.	0 tumors

De Coulon draws from this table the only possible conclusion—that arsenic plays no rôle in the production of tar cancer in mice.

With a tar that elicited no tumors in six mice after 100 days, though it was injected subcutaneously at a site which was repeatedly brushed with tar, Daels [61] produced no tumors in six other mice whose drinking water was mixed with solution of potassium arsenite (Fowler's solution).

Bierich [18] points out that the agent responsible for carcinoma of the bladder in aniline dye workers does not contain arsenic, and says that mice treated with arsenic and tar do not show the usual tar lesions of the skin, even after 120 days.[17] Indeed, he believes that when arsenic is administered internally to tarred mice the connective tissue acquires an enhanced resistance to encroachment by the epithelium, so that the development of cancer is actually delayed [19, 20] rather than facilitated.

E. Möller [189] has also expressed the belief that arsenic delays the development of tar cancer. She could discover no arsenic in a tar which was nevertheless actively carcinogenic, and, furthermore, no carcinomas developed among ten mice to which arsenic was subcutaneously administered during a period of 196 days.

Schiller [243a] likewise found that small doses of arsenic delayed, though they did not prevent, tar cancer in ten mice receiving intraperitoneal injections.

Mandl and Stöhr [176] painted 70 mice three times a week with a sample of coal-tar containing arsenic but saw no macroscopic changes in the skin even after seven months.

It thus appears to be fairly well established that arsenic plays no rôle of any importance in the initiation of tar cancer in mice, as tar devoid of arsenic will produce cancer, and tar containing it may fail. Whether arsenic actually will delay the development of cancer is not so clearly established, and there are those, in fact, who think that it hastens the process somewhat. Thus Itchikawa and Baum [111] say that while the addition of arsenic to the diet of tarred rabbits did not hasten the appearance of early lesions, the final transformation of early into fully developed carcinoma was accelerated.

The only similar observation is that of Ciechanowski and Morozowa,[56] and Ciechanowski, Morozowa, and Wilhelmi,[57] who assert, according to an abstract of their article, that arsenic greatly hastens the appearance of precancerous lesions. But their material comprises only twelve rabbits, six of which were given arsenic.

It is to be observed that those investigators who believe that arsenic retards carcinogenesis carried out their experiments on mice, whereas those who suggest an accelerating action employed rabbits. It may be that arsenic does exert some effect, and that this varies with different species, but experiments will have to be laid down on a larger scale if it is to be demonstrated. In the past a number of animals have been used, in some cases, such as an experienced investigator might consider, perhaps, for a preliminary experiment, but from which he would never venture to draw any conclusion.

THE TECHNIC OF APPLYING TAR

Duration of the Applications.—Although there may be minor differences in the length of time required to produce carcinoma with different strains of animals or with different tars, the periods reported by most investigators are essentially similar.

As Yamagiwa and Itchikawa [278, 279] have indicated, it is not easy to decide exactly when the carcinomatous change sets in. In their earlier experiments, carcinoma developed as early as the 103rd day in rabbits tarred every two or three days, and as late as the 565th. In a general review of their work, published in 1918, they [280] say that papillomatous new growths may be produced in from thirty to 100 days, and carcinoma in from fifty-five to 360 days. In most instances, 150 days or more was required.

Itchikawa and Baum [111] have given minute directions for obtaining malignant growths in the shortest time. They believe it necessary to paint a large surface, and advise applying tar to almost the whole internal surface of the ear, where the hair is thinner, watching carefully from the first to the fifth week for symptoms of tar poisoning. Although a general intoxication is regarded by them as a prerequisite, in order to diminish resistance to carcinoma, it is very dangerous to the rabbit, and during the period of tar poisoning applications should be made less frequently.

The frequency of the paintings should be decided by the reaction of the skin. Sometimes an acute eczematous condition, with erosions and vesicles, develops after the first tarring, in which case it is necessary to wait for a few days, until this reaction has become chronic, before proceeding with the applications.

When the tar has dried sufficiently to be removed with forceps, another application should be made; for it will be found that at this time the layer of tar is separated from the skin by dead surface epithelium and sebaceous matter. One should not wait, therefore, until the dried tar has fallen off spontaneously.

The authors recommend, as a general rule, three tarrings during the first week and two during each succeeding week, except that in the fifth only one is given.

By this method, fully developed carcinoma has been produced in forty-seven days, although this would appear to be a record, for the authors say that in twenty-four ears, early carcinoma has developed in twenty-two (92 per cent) at the eightieth day. The precancerous stage was attained in thirty-five days.

This corresponds closely with the thirty day period mentioned by Ciechanowski, Morozowa, and Wilhelmi [57] for the precancerous stage.

Gas-tar applied two or three times weekly produced small epithelial thickenings in rabbits in about six weeks, in the experiments of

Halberstaedter.[100] Some of these gradually increased in size and soon took on a warty character, while others regressed. Halberstaedter emphasizes the resemblance of these tar cancers of the rabbit to the roentgen-ray carcinoma of man and to the carcinoma sometimes produced by the sun's rays in sailors and farmers. There is, however, a difference between roentgen-ray cancer in man and tar cancer in the rabbit; for while the former requires years to develop, the latter arises within a few months.

It is likely that this is because of the greater life span in the human species, although Bloch [29] found that even in the rabbit roentgen rays require a much longer period than tar to produce carcinoma.

Von Witzleben [265] gives five weeks as the length of time necessary to produce tar warts in the rabbit.

A comparison of the foregoing figures shows that when tar is applied two or three times a week, papillomas may be expected to arise in from thirty to 100 days, and carcinoma in from forty-seven to 565 days. A period exceeding 150 days will usually be required for the production of a malignant new growth.

Not all painted rabbits, however, respond to tarring, and the investigator may very well have no carcinomas at all as the result of a long-continued experiment.

Soon after the publication of Yamagiwa and Itchikawa's epoch-making work, Tsutsui [262] showed that the mouse also is susceptible to tar; since then it has been employed in greater numbers than the rabbit, not only for economic reasons, but because it generally responds more readily to this irritant.

Tsutsui painted the skin of the back in mice with coal-tar every three or four days, and succeeded in producing cancer after 100 days or more. As in the rabbit, the tumors appeared first in the form of papules and continued to grow after the irritant had been discontinued. Two of the mice had metastases in the lungs.

Tsutsui's results were soon confirmed by Fibiger and Bang.[88] In mice painted every two or three days the first papillary outgrowth appeared on the eighty-sixth day, while all those that lived for from 257 to 331 days had malignant tumors.

In the following year, Fibiger and Bang [83, 89] reported that their earliest warts had arisen about four months, and the latest about eight months, after the first application, and that carcinoma had appeared in five months, or even earlier.

Lipschütz [159] found that the first papillomas appeared, in his experiments, after from 88 to 125 days.

In the experiment of Bierich and E. Möller,[24] Bierich,[21] and E. Möller,[189] papillomas appeared a little earlier. They produced hyper-

keratosis toward the end of the first month, papillomas toward the end of the second and beginning of the third, and carcinoma in the fourth month.

Murray and Woglom [100] found that when mice were tarred once or twice a week, nonmalignant tumors kept appearing from the fourteenth to the fifty-sixth week, although fifteen out of the seventeen observed had developed by the thirty-sixth week. The earliest carcinoma arose at the sixteenth week.

Bang [4] has published the following table to illustrate the importance of the duration of painting:

1 month's painting	14 mice	0 carcinomas
2 months' painting	16 mice	3 carcinomas
3 months' painting	13 mice	9 carcinomas
4 months' painting	12 mice	12 carcinomas

As mice painted for only one month escape cancer, whereas those painted for four months are almost certain to have it, Bang estimates that the duration of painting just necessary to produce malignant transformation is about two or three months. Rarely, however, does carcinoma appear until after four months' painting, and in most mice so treated it develops after six or seven months; the longest interval observed was 235 days.

When painting is discontinued after two or three months, cancer appears considerably later than it does in mice tarred for four months, the interval being about eight or ten months; the longest period observed in this group was 317 days, in a mouse painted for two months.

Deelman [64, 68] produced local thickenings of the skin in full-grown white mice painted three times weekly, in from ten to twelve weeks, and carcinoma in from three to four and one-half months. He ascribes this short interval to the unusual activity of his tar. In a later article, he [71] describes the appearance of cancer in from fifty to sixty days.

Murray [107] found that the length of time elapsing from the first tarring until malignant growth sets in may be as short as three or as long as eighteen months.

Truffi [260, 261] produced papillomas in from 115 to 150 days by painting mice every two to five days. He sets the shortest time within which malignant tumors were produced at 125 days, but adds that from 180 to 200 days or more may be required.

Foerster [92] used a horizontal-retort tar which was so active that infiltrating carcinomas were produced in eight weeks.

When the foregoing figures for the mouse are compared, irrespective of the frequency of application and the size of the area tarred, it will be seen that the time necessary for the production of carcinoma in the mouse is not very different from that required in the rabbit, in spite

of the longer life cycle characterizing the latter species. Thus the shortest period within which carcinoma has been achieved in the mouse is fifty days and in the rabbit forty-seven days. On the whole, however, the time for the mouse is about one month less than that for the rabbit; for while the period usually necessary in the rabbit is placed at 150 days or more, that for the mouse is pretty generally agreed to be in excess of 120 days.

The maximum for the mouse is 540 days and for the rabbit 565 days.

Curiously enough, papillomas may develop after a shorter period of tarring in the rabbit (thirty days) than in the mouse (sixty days), and the maximum time after which papillomas have appeared is also shorter in the former species—100 days, as compared with 392 days for the mouse. It is to be remembered, however, that a much greater number of experiments have been carried out with the mouse than with the rabbit.

Size of the Tarred Area.—The size of the area painted and the frequency of the applications may, perhaps, have some influence on the yield of tumors or the time necessary for their development, although in order to demonstrate this conclusively a large number of mice of the same strain would have to be painted with the same tar; for different breeds of mice and different samples of tar at the hands of different investigators cannot be safely compared.

It has already been said that Itchikawa and Baum [111] suggest the painting of a wide surface in the rabbit, and Deelman [69] believes that tarring of a large area will hasten the appearance of carcinoma in the mouse also. Murray,[197] on the other hand, is not convinced that the size of the area painted has much influence, and points out that whereas Deelman painted the whole dorsal surface of the animal, Fibiger and Bang a square centimeter, and Murray and Woglom much less, all achieved about the same results in the end.

Interval Between Tarring.—Deelman,[71] who has investigated the effect of the intervals elapsing between successive tarrings, comments on the remarkable similarity of the figures representing the number of applications required to produce macroscopic changes in the skin, in seven series of fifteen mice each. Twenty-two applications were required when tar was applied at intervals of two days; eighteen applications when it was applied at intervals of three days; nineteen applications with four day intervals; seventeen with five or six day intervals, and eighteen when painting was carried out every seven days.

Furthermore, cancer appeared in from fifty to sixty days—an almost constant period—although the total time during which tarring was carried out ranged from forty-four to 140 days. It is evident, he says, that the development of carcinoma depends on a summation of the irritations, but, also, that when this cumulative effect has reached a certain point the malignant transformation proceeds inexorably, whether the irritation is continued or not.

Cessation of Tarring.—It is agreed by all who have worked with experimental tar carcinoma that malignant tumors will arise after a certain period of tarring, whether the irritant is continued or not.

Leitch,[150] who has made a special study of this question, said that if tar painting in mice is stopped after warts have developed, some of these lesions will disappear, some will grow for a time and then regress, some will continue to increase in size but remain simple warts, and others will go on to malignancy, probably as soon as if the irritant had been continued. From these observations he drew two conclusions: 1. Production of the cancerous state is not necessarily a result of action by the irritant on neoplastic as contrasted with preneoplastic tissues. 2. The bias toward malignancy is probably given the cells during the preneoplastic stage.

In a supplementary experiment he painted twenty mice thrice weekly for from four to five months, suspending the applications before there was any sign of a papilloma. Six remained negative, four developed temporary warts, four simple papillomas and six had malignant tumors. Thus tar appears to produce some profound change which cannot be detected by the microscope, and which eventually results in malignant proliferation, even though irritation has been discontinued in the meantime.

Leitch's conclusions are amply confirmed by the work of Findlay.[91] This investigator made one application of hot tar (70 C.) in seventy-five mice; 341 days later three of the thirteen survivors had developed epitheliomas at the site of the tarring, which grew progressively and resembled both macroscopically and microscopically those produced by the long-continued application of tar.

The Latent Period.—Bang [3-7] calls the time which elapses between the first tarring and the occurrence of invasive growth the "developmental period," and divides it into a "preparatory period," which lasts for two or three months, or until potential malignancy sets in, and a "latent period," which runs from the end of the preparatory period up to the supervention of invasive growth. Thus in mice painted for four months only, infiltration by the epithelium is rarely found at the end of this time, yet during the following months cancer appears. It is evident that a latent cancer is present, even though no alteration in the appearance of the epithelium other than a slight hyperplastic change can be discerned under the microscope.

The duration of the latent period in the mouse is set by Bang at from eight to nine months, or about one third of its life span. This would correspond to twenty or thirty years in man, and Bang points out that chimney sweeps and aniline or paraffin workers often develop cancer this length of time after having changed their occupation.

He believes that the latent period thus observed in experimental tar cancer helps to explain the tendency of carcinoma to arise in later life, and suggests that the disease may not be due to senile changes in the tissues, but to some extrinsic factor that requires a long period to exert its effect.

Asking themselves whether the latent period might not be shorter if malignant change were the only factor concerned, and the body were not combating an incipient cancer by some such means as immunity, Sachs and Takenomata [242] inoculated tarred mice with a transplantable carcinoma. The experiment comprised four groups of mice that had been painted for from ten to ninety-seven days. Of twenty normal controls, nineteen were positive to inoculation (95 per cent) and one was negative, whereas of twenty-one tar-painted mice thirteen were positive (62 per cent) and eight negative; furthermore, tumor growth in the tarred mice was not so vigorous as in the controls.

I must confess that I have not been able to grasp the significance of this experiment. It has been shown by several investigators in the past that spontaneous neoplasms can arise in animals totally immune to propagable tumors, so that even if the tarred mice in the experiment had been entirely refractory to the transplanted carcinoma, which is far from being the case, it could not be inferred that they would show any resistance to the initiation of a tar carcinoma. And again, the authors suggest that the cells of the developing tar carcinoma immunize the mouse, although Woglom and others have shown that the mouse cannot be immunized with its own tissues, and Haaland has demonstrated that it cannot be immunized against its own tumor.

A much simpler explanation for the slight resistance observed in the tarred mice is tar poisoning, for it is known that tar painting often makes animals seriously ill, and that tumors grow best in those which are healthy.

Modification of the Tarred Area.—Attempts have been made to shorten the latent period and increase the tumor yield by modifying the painted area. A certain amount of mechanical injury had been added to the chemical stimulation of the tar in the experiments of Yamagiwa and Itchikawa and of Tsutsui, who removed with forceps the layer of dried tar resulting from the previous application. But Deelman [68, 70] advocated a slightly more extensive trauma in the form of light scarification carried out before the first few applications of tar. By such means he was able to produce cancer in the mouse in five weeks. Microscopic study showed that tar penetrated the scarified skin down to the deepest layer of the epidermis, or that it might even reach the derma, and he ascribed the shorter period required for the initiation of malignant growth to this intimate contact between the tar and the tissues.

Deelman's observations were confirmed by Teutschlaender,[256] and Reding [219] reported that he had been able to elicit cancer in from seven to nine weeks in almost 100 per cent of his mice when tar was applied three times a week preceded by scarification. He issued the warning, however, that in mice so treated the mortality is high.

Other investigators, on the contrary, have not been so successful in verifying Deelman's results. Thus Mandl and Stöhr [176] failed to produce carcinoma in mice which had proved refractory to fourteen months of tar painting, and which were then scarified and painted for two months more.

Truffi [260] found that, although trauma seemed to accelerate the appearance of papillomas, it did not hasten their progress to carcinoma.

Roussy, Leroux, and Peyre [235, 238] scarified mice by several methods, but did not achieve the quick results described by Deelman when they employed a French tar known to be carcinogenic. And even when Deelman's own tar was used, the results were no better. Ninety mice in all were scarified and painted, but among eighteen that survived for 170 days not one tumor developed; nor did four that lived for 250 days have tumors at the end of this time.

Petit [215] remarks that it is not necessary to scarify, as by scratching the tarred area the mice perform this operation themselves more delicately than the investigator can do it.

Daels [61] tried the effect of a more severe traumatism than that recommended by Deelman. He soaked threads in tar or other irritants, passed these into the skin of mice and allowed them to remain for from fifteen to twenty-five days; at the end of this period another one was inserted, and so on, the irritant being kept in this way constantly in contact with the wound. In other experiments, a strip of skin was removed, painted with tar, and buried subcutaneously, while in still another group of mice sutures were passed into the skin after it had been cauterized twice in six weeks with a dull cautery. The yield of tumors was much better in controls that were simply painted with tar, whence the author concludes that cicatricial transformation of the subjacent connective tissue reduces the disposition of the epidermis to neoplastic change.

Derom [77] heated an epilated area in mice with a lead shield, raised by immersion in water to a temperature of 50, 60, or 70 C. The thickened and scarred skin resulting from application of the shield at 60 or 70 C. was more resistant to tar than normal skin. Although the number of mice is small, the outcome agrees with Parodi's statement [206] that preliminary burning does not favor the development of tumors. In Derom's control group there were twelve tumors in twenty mice, or 60 per cent; in the 60 C. group, ten mice with four tumors, or 40 per cent; and in the 70 C. experiment, thirteen mice with five tumors, or 38 per cent.

On the contrary, when the skin was heated to only 50 C., the appearance of tumors was hastened, and among eleven mice there were seven tumors, or 64 per cent. Although there is a difference of only 4 per cent between this lot and the controls, the author concludes that the application of a mild degree of heat (50 C.), which causes neither macroscopic nor microscopic changes in the skin, confirms the results of Deelman.

Exposure of the skin of mice before tar painting to either filtered or unfiltered radium, does not hasten the development of cancer, according to Roussy, Leroux and Peyre.[238] The roentgen ray, on the other hand, is said by Domagk [79] to increase greatly the yield of tumors when applied in small doses.

Following the suggestion of Blumenthal,[33] that the permeability of the skin for tar might be increased by rubbing with petroleum ether, Rémond, Sendrail, and Boulicaud [223] tried a preliminary cleansing with this fluid, or with xylol, as suggested by Roussy. The procedure, however, did not seem to hasten the appearance of tar cancer in rabbits.

The outcome does not correspond with the experience of Bittmann [26] in the same species of animals. Bittmann chose fourteen rabbits from different sources, so that any predisposition of one particular strain might be avoided, and rubbed the ear with a pledget of cotton soaked in petroleum ether for five minutes; after a five minute interval, tar was applied, the procedure being repeated every two days. In the eight rabbits that survived, verrucous effloresences developed after from fourteen to thirty-six days, which microscopic examination showed to be already of an invasive character. Outward growth, as in a wart, was not seen, the lesions appearing to be invasive from the first. Lateral growth and ulceration followed, and in the course of time invasion of the cartilage. There were metastases in the regional lymph-nodes after from six weeks to three months.

The same rapidity of development has been described by Bartozek [9] in the mouse, when the skin was cleansed with ether before the tar was applied. Tumors appeared after sixty-eight days, as compared with 103 days in the controls.

There are substances which appear not to hasten but to delay carcinogenesis, although the number of experiments is still small. Reding [219] suggests that the implantation of fragments of metallic magnesium beneath the skin may delay the appearance of tumors by about one month, but there were only seven mice in the experiment—four treated and three controls. The subcutaneous introduction of colloidal magnesium or of a mixture of colloidal calcium and magnesium sulphate seemed to prevent the development of tumors, the skin remaining smooth and soft; but here again the number of surviving mice was small, and the author does not wish definite conclusions to be drawn

until he has had opportunity to repeat the work on a sufficiently large number of mice.

According to Lecloux,[146-147a] the application of a solution of sodium oleate between tarrings delayed for from thirty to fifty days the appearance of papillomas in mice. Once the tumors had appeared, however, cancerization was as rapid as in the controls, although the resulting carcinomas did not grow so rapidly. There was no effect exerted on tumors already established.

The inhibiting agent appeared to be the acid radical, not the metallic ion, for oleic acid had the same effect. Two other 18-carbon acids, linoleic and stearic, differing only in the degree of saturation, had not given definite results at the time that the paper was written.

Modification of the Tar.—The modifications so far applied to tar have been two in number—heating and electrolysis.

Derom [77] found that a higher percentage of tumors could be produced in mice, and that they appeared in a shorter time, when tar was applied at a temperature of 70 C. than when it was used at 60, 50 or 37 C. or at room temperature.

Rémond, Sendrail, and Boulicaud [223] have reported that tar employed at 56 C. has a prompt carcinogenic action in rabbits, but lower degrees of heat (from 45 to 50 C.) are ineffective in the same species, according to Ciechanowski, Morozowa, and Wilhelmi.[57]

A curious and interesting observation has been recorded by Kotzareff and de Morsier.[141] The ears of three rabbits were tarred six times at two hour intervals on the first day, once daily for the next three days, every second day for three or four weeks, and then twice weekly. One of the three was painted with ordinary coal-tar. In the case of the other two, a current of 20 volts was passed through the tar for an hour just before it was applied, and the left ear of each rabbit was then painted with tar from the positive pole and the right with tar from the negative pole.

In the control animal, painted with ordinary tar, there was only an epithelial hyperplasia on the fifty-third day, whereas the two rabbits painted with electrolyzed tar had developed cancer on the sixteenth and seventeenth days, respectively.

The experiment was repeated by Kotzareff,[140] a current of 110 volts being passed through the tar for one hour before the first application was made, and for ten minutes before each succeeding application. The rabbits and tar were from Geneva, yet the same acceleration was observed as in the first experiment with rabbits from Constantinople and tar from Paris; tar from the positive pole produced early cancer in seventeen days, while that from the negative pole elicited a more advanced cancer in nineteen days. The control rabbit, painted with nonelectrolyzed tar, had only an epithelial hyperplasia on the twenty-third day.

The diagnosis of these tumors was confirmed by Itchikawa.

As a tentative explanation, Kotzareff suggests that the electric current may dissociate large molecules in the tar, so that passage through the cell membrane becomes possible.

Peyre and Kotzareff [213] carried out this experiment a third time, with still another sample of tar, and again noted the appearance of early carcinoma on the seventeenth day. As in previous experiments, tar from the negative pole appeared to be even a little more active than that from the positive.

The authors emphasize the difficulty of drawing conclusions from the facts reported, because the number of animals is small, the result of electrolyzing tar obscure, and finally, the diagnosis of cancer in its early stages has to be made on the basis of minute histologic differences.

Tar Injections.—In order to obtain tumors which would be unulcerated, and therefore possibly transplantable, Yamagiwa and Itchikawa [281, 282] tried injecting tar into the breast of the rabbit, and they succeeded in producing carcinoma by introduction of a mixture of tar and hydrous wool fat. These experiments were continued by Yamagiwa, Suzuki, and Murayama,[286] who reported a fibromyxosarcoma after the injection of tar and hydrous wool fat, or of tar alone, into the breast of a female rabbit once or twice a month for twenty-three months, and by Yamagiwa and Murayama.[283-285] In a recent review of their investigations, the authors last named report on the results of injecting tar or tar mixtures in doses of 0.3 to 0.5 cc. once or twice a month.[285] In 188 rabbits, twenty-three (12 per cent) cancroids or adenocancroids were produced. As the glandular epithelium of the breast does not respond to tar, only cancroids from the ducts, hair follicles or surface epithelium were obtained, or adenocancroids from the ducts.

They say that hydrous wool fat, which Japanese investigators so often add to tar, does not expedite the appearance of malignancy, but merely causes a local lipoidosis which facilitates invasion once the epithelium has become malignant.

The rabbit was chosen by Seedorf [248] also. Among thirty-nine injected in the breast every three weeks, sometimes with coal-tar alone, sometimes with coal-tar mixed with hydrous wool fat, eight survived for one year, but none developed a carcinoma, though the tar was that used with success in mice by Fibiger and Bang.

Ishibashi and Ohtani [107] introduced 0.5 cc. of coal-tar into the submucosa of the stomach or intestine of rabbits, and two weeks later found papillary adenomas, which after fifty days had shown no evidence of regression, in the stomach. In the intestine there was nothing but a slight hyperplasia, with no sign of adenomatous proliferation.

Ibuka [106] produced atypical epithelial growth in the lungs of three of fourteen rabbits by introducing bits of paraffin impregnated with soot into this organ. The animals lived, however, only for fifteen, fifty-three, and sixty-eight days, respectively, too short a time for tumors to develop.

According to Leitch,[154] rectal injection of tar in rabbits produced papillomas but no carcinomas.

Kimura [138] introduced coal-tar into the bronchi of rabbits and guinea-pigs under anesthesia, through a tracheotomy wound. One rabbit of three survived, and was killed on the eightieth day; in the lung there was found an encapsulated microscopic area composed of tortuous tubules lined with one layer of epithelium, which the author regarded as an adenoma-like growth. Three guinea-pigs surviving out of ten were killed on the 140th day, and in the lung of one there were found several adenocarcinomas, one of which contained a fragment of bone.

Lacassagne and Monod [145] made two intratesticular injections of from 1 to 5 drops of tar, at fourteen day intervals, into five rabbits, three of which were living when the article was written. One had died ninety-eight days after the first injection, without any lesion of consequence; but the other, killed 355 days after the first injection, had a sarcoma of the testis, which showed invasive growth but which had not metastasized and did not grow after transplantation.

Few authors have injected tar into the mouse. Seedorf [248] introduced it into the breast of seven white female mice in an endeavor to produce a glandular carcinoma, and 186 days later discovered a small subcutaneous nodule in one of them. Biopsy on the 301st day disclosed an adenocarcinoma of the type so commonly encountered in mice. This was successfully transplanted, and when the original mouse died, on the 323d day, metastases were found in the lymph-nodes and lungs. This experiment is not very convincing, as the tumor resembled the ordinary spontaneous carcinoma of the mouse breast.

In order to discover what effect tar would have on the connective tissue, Russell [240] administered weekly subcutaneous injections of from 10 to 15 mg. of tar to fifty male mice three or four months old. Nine months later, one of the seven survivors had a small nodule, which grew slowly until it was excised two weeks later and transplanted into the mouse itself and into other mice. When the animal died, two weeks after operation, the autoplasts had grown, but no metastases were detected at necropsy.

The grafts in other mice proliferated slowly, in a low percentage of inoculated animals, and the tumor was cultivated through six generations; but, showing no improvement in growth, it was then allowed to die out. No other tumors developed in the remaining six animals, the last of which died thirteen months after the beginning of the experiment. The tumor was a spindle cell sarcoma.

Percentage of Success.—Although one of the most important of all the questions which arise in connection with experimental tar cancer is the proportion of animals susceptible, this is at the same time one of the most difficult to answer. The percentage of success reported, which runs all the way from 0 to 100, varies with the tar used, the strain of animal employed, and with the enthusiasm and experience of the investigator. While some observers are careful to lay down rigid qualifications which any given lesion must satisfy before being classified as malignant, others are so lenient in this regard that the reader cannot but wonder whether the proportion of carcinomas was actually as high as is asserted. In other cases, again, a diagnosis has been made solely on the macroscopic character of the lesion, or, even though a careful microscopic examination has been made, the percentage of success achieved has been reckoned on but five or six animals.

In any case, the percentage of success is much higher in the mouse than in the rabbit, no investigator having reported 100 per cent of success in the latter species, so far as I am aware. Yamagiwa [271] says that in the earlier experiments which he carried out with Itchikawa the percentage was relatively low, only twelve carcinomas having been obtained in about 200 animals (6 per cent, approximately). In later experiments,[270] 15 per cent was reached. When rabbits live for 300 days or more, however, it is possible to achieve over 30 per cent, according to Itchikawa,[109] or even 77 per cent if especial care be taken of the health of the animals in order that they may survive long periods of painting.

In the mouse, Tsutsui [262] obtained 24 per cent of carcinomas, or sixteen in sixty-seven mice that lived for more than 100 days. Among subsequent investigators, the palm for low percentage must be awarded to Renaud,[225] who tarred 130 mice for seventeen months without obtaining a single carcinoma. The next lowest percentage is probably that of Dentici,[75] who succeeded in producing carcinoma in only two of eighty-six mice; the tumors appeared at the 239th and 252d days after tarring was begun.

On the other hand, Fibiger and Bang [88] produced malignant tumors in all six mice that survived for from 257 to 331 days, in a group originally numbering fifteen; and in the following year Fibiger [83] reported a series of forty-five white mice, of which all twenty-six that survived painting for from 180 to 397 days had papillomas by the 180th day. Three of the twenty-six died soon afterward, one of which had developed a carcinoma in the meantime, and the remaining twenty-three, some of which lived for 397 days, all had malignant tumors. This would mean that from 92 to 100 per cent of the mice finally had malignant growths, according to whether or not the two that died with simple papillomas soon after the 180th day are included in the reckoning.

Deelman,[68] Teutschlaender [257] and Maisin and Desmedt [173a] also find that all mice which survive long enough will develop cancer, although ideas vary in respect to how long a period is long enough. Thus Teutschlaender says that every mouse that lived more than four months developed cancer in his experiments, whereas Maisin and De Smedt base their statement on a survival period of seven months.

The reader will now have some appreciation of the difficulty of deciding what proportion of animals exposed to an irritant will ultimately develop malignant new growths. For in various experiments throughout the world the tar has differed, the strain of mice has differed, and the method of calculation has differed. It seems probable, however, that given mice of ordinary susceptibility and an average sample of tar, an investigator could reasonably expect to obtain about 70 per cent of tumors among mice that survived for at least four months.

Among the few who have discussed this question, Borst [40-42] is particularly skeptical of the achievement of 100 per cent of tar cancers. He emphasizes the difficulty that even the most experienced pathologists have in distinguishing atypical epithelial growth from carcinoma, and cites the disappearance of tar tumors of the rabbit, which, on microscopic examination of fragments removed at biopsy, appeared to be typical carcinomas. He will not admit the diagnosis of carcinoma unless infiltrative growth and metastasis can be definitely proved.

Murray and Woglom [199] made use of four criteria of malignancy: (1) the progressive growth of a tumor after painting has been discontinued, and the growth of its autoplasts; (2) recurrence after wide excision; (3) histologic evidence of local infiltrative growth, and (4) metastasis.

They regarded loss of differentiation and the presence of atypical cellular characteristics as wholly inadequate, nor did they employ transplantation into other animals as a test for malignancy, for it is well known that many spontaneous mouse tumors, particularly those of the squamous cell type, either fail to grow in normal animals or take in such a low percentage of cases that the test would have to be carried out with inordinately large numbers of mice. If the test were negative, malignancy would not be excluded, and if positive, the result might be equivocal by reason of a merely temporary proliferation of the grafts.

On the other hand, inoculation of a tumor into the affected animal itself is almost always successful, and autoplastic rather than homoplastic transplantation was therefore chosen to test the character of these tar tumors. After excision of a tumor, fragments were removed from the edge in its deeper part, and immediately introduced subcutaneously into the right and left flanks of the bearer, the remainder of the growth being preserved for histologic examination.

As normal tissues and benign growths proliferate only temporarily or not at all on autoplastic transplantation, the authors expressed the belief that growth of the autoplasts could be confidently regarded as evidence of their malignant nature.

In twenty mice inoculated with their own tar tumors, the autoplasts grew in fifteen and remained stationary in one, probably because the animal was suffering from a chronic general infection which ultimately proved fatal. In three of the four remaining mice the grafts proved to be infected, and ulcerated out, and in only one instance did the autoplasts remain in situ in an apparently healthy mouse and fail to grow. Thus when noninfected grafts were inoculated into healthy animals, there were fifteen successes in sixteen attempts.

An interesting by-product of these autotransplantations was the observation that a tar tumor might be transplantable before it had begun to exhibit invasive growth, two such instances having been encountered.

The article terminates with the conclusion that the progressive growth of an autoplast is a proof of malignancy, even though the tumor may appear to be benign, and the suggestion that some of the lesions regarded by preceding investigators as benign might have proved malignant had they been tested by autoplastic transplantation.

The hope of the authors that more extended use might be made of this convenient test for malignancy has not been entirely fulfilled, however; partly because, as in the experiments of Fukuda,[94] the autoplastic transplantation has not been successful, and partly because other investigators, like Leitch,[150, 152] do not think that it yields any additional or more definite information. Leitch believes that the successful transplantation of a tumor in the histologically doubtful stage is not a certain proof of its malignancy at that particular date, since it might have declared itself malignant ultimately had it been left in its natural environment.

Kreyberg [142] was unable to confirm the observation of Murray and Woglom that a histologically benign tumor might prove to be transplantable. Among his twenty-five tar tumors of the mouse, ten were histologically benign, and inoculation gave negative results in all; two of doubtful nature gave negative results; while of thirteen that were histologically malignant, ten could be successfully inoculated by autoplastic transplantation. Infection of the graft accounted for the three failures. Thus Kreyberg found a complete parallelism between the histologic structure of an autoplast and its behavior on autotransplantation.

Bang,[5] on the other hand, agrees with Murray and Woglom that a tissue may be malignant without showing any morphologic evidence of its condition. And Roussy, Leroux, and Peyre,[233] in discussing the uncertainties of diagnosis, said that a decision is sometimes impossible. They regarded malignancy as a biologic rather than as an anatomic concept.

THE CONSTITUTIONAL EFFECTS OF TARRING

The production of a tumor is not the only way in which tarring injures the organism. The external application of tar is followed by serious poisoning, more profound with some tars or with some fractions than with others, yet hardly ever negligible, so that it is necessary for the investigator to begin his experiment with hundreds of mice, rather than with dozens, if he expects to have a sufficient material for conclusions at its termination.

Tar probably gains access to the body both through the skin, its entrance being facilitated by the scratching of the animal, and by way of the gastro-intestinal tract during the efforts of the animal to cleanse itself of an irritating material, as will be shown in a later paragraph.

As a proof of the profound constitutional effect exerted by tar, Maisin and De Smedt [173] mention the rarity of pregnant mice in a tarred group. Sterility appeared to set in at about the second month of painting, and to persist for two months after the applications had been discontinued. Nevertheless, the animals were not cachectic.

On the other hand, Lecloux,[146] who employed a tar of equal carcinogenic power, found it so mild in its constitutional effects that hardly a mouse died during the experiment and tarred females went through several normal pregnancies. Parodi,[206] too, said that tarring does not interfere with reproduction in mice.

In the rabbit, Krotkina [143] has observed six pregnancies in two tarred animals.

These diverse reports are but additional evidence that tars differ considerably in their action, and that it is impossible to generalize from the results of one investigator. Yet there can be no doubt that many tars are extremely toxic and that their external application is followed by anemia, cachexia, and death, as Lipschütz [160, 165] has indicated. He found an intense vascular proliferation in various organs, extramedullary blood formation, lesions of the reticulo-endothelium, icterus, degenerative changes in the kidney and liver, and the deposition of large quantities of blood pigment in the spleen and lymph-nodes.

Bierich [21] mentions leukocytosis, with albumin, erythrocytes and sometimes casts in the urine of mice painted with tar in which necropsy disclosed toxic nephrosis.

Mertens [185] also encountered nephroses, as well as fatty degeneration with hemorrhages in the liver, and inflammatory lesions in the lung which sometimes advanced to abscess formation.

In the lungs, Dentici [74, 75] found simple hyperemia or even bronchopneumonia. The kidneys were the seat of hyperemia and early degenerative lesions, and the liver of more or less advanced degeneration with perivascular infiltration, while the spleen and the lymph-nodes showed follicular hyperplasia or, later, sclerosis.

Paszkiewicz [212] has described parenchymatous lesions of the liver, a serous exudate in Bowman's capsule and the convoluted tubules, fragmentation of the myocardium, fibrosis of the splenic capsule and trabeculae with atrophy of the lymph-follicles and thickening of the follicular vessels, and the presence of red blood cells and hemosiderin in the splenic pulp.

Hyaline degeneration of the spleen has been observed in tarred mice by Fukuda and Azuma,[95] myeloid metaplasia of this organ combined with an increase in its blood pigment by Mandl and Stöhr,[176] lymphocytosis and renal lesions by E. Möller.[189]

The changes in the spleen, liver and kidney cited by various observers have been confirmed by Polettini.[218]

The blood of tarred rabbits has been exhaustively studied by Itchikawa and Baum,[112] and Itchikawa, Nakahara, and Uwatoko.[121] They found that the number of red blood cells and the percentage of hemoglobin gradually diminish as tar cancer develops, while the number of granular leukocytes increases. The lymphocytes remain at the normal level if the animal is in good health, but diminish in number if it begins to lose weight. As the blood changes do not set in until the approach of malignancy, the authors are inclined to ascribe them to the cancer rather than to the tar.

In connection with these lesions of the various organs, the tissues of painted animals have been searched for tar, and granules of this material have been found in the spleen, lymph-nodes, liver, lung and kidney by Dentici,[74, 75] Mertens,[184, 185] and Roussy, Leroux and Peyre.[233, 236] Dentici has traced its passage into the circulation by way of the lymph-channels, and obtained direct proof of its absorption into the body by inspection of the peritoneal lymphatics and by the discovery of tar granules in histologic preparations of the viscera.

He was able, furthermore, to demonstrate these granules in the basal layer of the skin, where they appeared as minute, rounded, blackish, translucent granules which did not give an iron reaction and which were often intracellular or contained in phagocytes.

Tar granules have been described in the skin by Bierich [17] also, who found them between the epithelial cells and in the subepithelial layer, as well as by Roussy, Leroux, and Peyre.[233] Peyron,[214] however, asserted that the latter authors had mistaken the granules of mastocytes (the mast cells of German histologists) for tar granules, to which Roussy and his collaborators replied [236] that other observers had also seen these tar granules, and that they were visible in unstained preparations. This did not quite settle the question, however, for in the following year Itchikawa and Baum [113] suggested that the tar granules observed by Bierich, and by Roussy, Leroux, and Peyre were probably blood pigment.

Constitutional effects less serious than tar poisoning have been communicated from time to time, such as a generalized change in the sebaceous glands mentioned by Lipschütz [160] and verified by Mertens,[185] in the course of which these structures undergo cystic dilatation and are converted into small, yellowish, raised nodules.

Still another outcome of tarring in mice is said by Silberstein, Freud and Révész [250] to be a respiratory quotient which remains strikingly constant in spite of the diet. Such animals are killed by one half to one sixth of the usual lethal dose of insulin, and the authors affirm that dogs painted with tar exhibit the same susceptibility to it.

Harde [102] found that the application of tar has no effect on the paratyphoid agglutinins of rabbits, but that it suppresses the reaction to vaccine virus. A similar modification was noted in mice, although it was explained by the author that even in the normal mouse the response to the virus is not so pronounced as in the rabbit. He raised the question whether this diminished sensitivity of the skin is due solely to the tarring, or whether precancerous cells are less responsive.

As the virus was sometimes applied in the flank, whereas it was the neck and ears that were tarred, the latter explanation appears improbable.

Finally, the question whether tar painting increases or decreases susceptibility to a transplanted tumor has been approached by several investigators. It has already been mentioned in another connection that Sachs and Takenomata [242] observed a certain resistance in tarred mice. Though this has been confirmed by Mandl and Stöhr [176] on a small number of mice, the experiments of Parodi (cited by Truffi [259]), Polettini,[217] and Murphy and Maisin [193] suggest rather that tarring increases susceptibility. The question cannot be regarded as settled, however, for the number of mice concerned in the latter experiments was small, and none of the authors desired to draw definite conclusions from an admittedly inadequate material.

THE LOCAL EFFECTS OF TARRING

Macroscopic Lesions.—Yamagiwa and Itchikawa,[277, 279, 280] who have given a careful account of the local lesions which attend tarring of the rabbit's ear, recognize the following four periods:

1. Atypical Growth of the Epithelium. About a week after applications of coal-tar have been begun, the epithelium, especially that of the hair-follicles, appears hyperkeratotic and cystic dilatation with keratin retention sets in, while at the same time the hair begins to fall out. These changes become gradually more distinct over a period of from 30 to 360 days.

2. Appearance of Folliculo-Epithelioma. About fifty days after irritation has begun the epithelium of one or more hair follicles is

hyperkeratotic to a high degree. It becomes gradually more and more elevated, and eventually there develop papillomatous new growths, of which there may be twenty or more; these are variable in shape and size, although roughly divisible into two types—*pedunculated* and *sessile*. If the interstitial connective tissue proliferates also, the growths resemble fibro-epitheliomas or fibromas. On the cut surface of all there is visible a number of dilated hair follicles in which keratin has accumulated. More and more of this substance collects in the enlarged follicles, until finally these tumors come to resemble the cutaneous horns of the human subject. If they are broken off at the base, new horns grow again at the same site or from the contiguous epithelium.

3. Production of Carcinoma. In the course of continued tarring the cut surface of some of these lesions becomes so irregular that one hair follicle can no longer be distinguished macroscopically from another. The surface of the new growth ulcerates at about this time, the tumors increase in size, and the walls of the ulcers gradually thicken so that the lesions begin to resemble rodent ulcers. Certain of these neoplasms continue to grow until they imitate even macroscopically the carcinoma of man.

4. Metastasis. The regional lymph-nodes often swell, and in such cases metastatic deposits may be demonstrable.

Not all tar tumors in the rabbit, however, grow progressively; on the contrary, a great many of them recede spontaneously, as they do in the human subject.

Tar lesions in the mouse, first announced by Tsutsui,[262] do not differ essentially from those just described. Like those of the rabbit, they may be papillomatous or sessile, and are likely to be multiple. Either type may remain solitary, or lesions nearby may fuse. The pedunculated type may drop off, leaving a carcinomatous ulcer.

The work of subsequent investigators has not added anything of importance to these first accounts of the gross appearance of experimental tar carcinoma.

Microscopic Lesions.—Yamagiwa and Itchikawa [275, 277, 280] also published a description of the microscopic changes that follow tar painting in the rabbit, dividing the process into four stages in order that it might correspond with their account of the gross lesions.

1. Atypical Growth of the Epithelium. The epithelium, and especially that at the periphery of the hair-follicles, gradually undergoes hyperplasia; each layer increases considerably in thickness, many mitoses are to be found in the basal layer, the hair-follicles become cystic, and the outline of the basal layer grows irregular from projecting processes which ramify in the subjacent subcutaneous tissue. The blood vessels, especially the veins and capillaries, soon dilate, while eosinophils and lymphocytes escape into the connective tissue.

2. Appearance of Folliculo-Epithelioma. The papillomatous elevations on the irritated surface, which have already been described in gross, consist of one or more hair-follicles in which hyperplasia and hyperkeratosis are present in high degree, the process resembling that seen in the preceding period except that it is more advanced. From these elevations there arise new growths, which the authors divide into two general types—*pedunculated folliculo-epithelioma* and *sessile folliculo-epithelioma.*

The pedunculated variety arises when hair follicles in the central or the peripheral regions of an elevation are lifted upward by the pressure of epithelial growth and continue to proliferate. As these neoplasms develop primarily from the epithelium of the hair follicles, the authors prefer to call them folliculo-epitheliomas rather than papillomas. In their subsequent growth, some of these pedunculated folliculo-epitheliomas undergo an increase in their connective tissue, thus becoming fibromatous; others come to resemble the sessile type, and still others develop into cutaneous horns.

Sessile folliculo-epitheliomas arise from one or more hair-follicles when the epithelium of an elevation undergoes a higher degree of hyperplasia and hyperkeratosis. The cystic hair-follicles are arranged almost directly on the ear cartilage, and the epithelium and corium extending upward between them form septums, the spaces between which are filled with keratinized epithelial cells. Hence these neoplasms have wide bases and are elevated but little from the surface of the skin, although occasionally one or more hair-follicles may push upward as in the pedunculated lesion; and some of these sessile folliculo-epitheliomas develop into cutaneous horns. The blood vessels become greatly dilated, eosinophils and lymphocytes are found in the connective tissue, and the basal layer of the epithelium contains a large number of mitotic figures. Mucoid degeneration of the connective tissue is not a characteristic of folliculo-epitheliomas.

3. Production of Carcinoma. This period Yamagiwa and Itchikawa divided into three stages.

(*a*) The earliest stage of carcinoma. It is difficult to distinguish these very young carcinomas from benign folliculo-epitheliomas, although the authors suggest that the following features may be of service. Some or all of the epithelium assumes a fainter stain with hematoxylin than does normal epithelium or that of the benign folliculo-epitheliomas; the budding processes which develop by atypical proliferation of the basal epithelium of the hair follicles grow more angular at their outer edge and very irregular in thickness; the interstitial connective tissue becomes loose in structure or shows a slight mucoid degeneration; lateral and downward penetration of the epithelium can

be demonstrated. Such changes as these are regarded by the authors as representing the initial step in malignant transformation.

(*b*) The early stage of carcinoma. By prolonging the applications of tar, more advanced changes than those just cited can be produced. In such an event the connective tissue becomes loose and edematous, the sprouts from the hair-follicles grow still more angular and more irregular in thickness, lateral and downward invasion takes place to a higher degree than in the earliest stage and the invading processes fuse with one another to form an epithelial network; the intercellular spaces become wider, and individual cells leave the main group; the epithelium takes a fainter stain with hematoxylin than does normal epithelium or that exhibiting simple atypical growth; dilatation of the capillaries and veins is generally more advanced than in the earliest stage; and invasion of veins or lymph-channels may occur.

(*c*) Fully developed carcinoma. The features distinguishing the two previous stages are more distinct, especially infiltration by the epithelium, which now becomes really striking. Its cells penetrate more vigorously into the connective tissue, enter the veins and lymph-channels, and even push through the aural cartilage to form ulcerating new growths on the opposite side of the ear. Mucoid degeneration of the connective tissue is noticed in almost all cases of fully developed carcinoma.

4. Metastasis. Lymph-node metastases may be solid or cystic; in the latter case the cyst-wall is lined by multilayered epithelium, which may infiltrate the lymph-sinuses and the surrounding tissues.

Tsutsui's description [262] of the microscopic features of tar carcinoma in the mouse was not so detailed. In his experience also, the papillary tumor was not so likely to exhibit invasive growth as the sessile variety. He did not mention participation by hair-follicles in the malignant process, but said merely that the basal layer of the epithelium assumes an invasive type of growth. The reason for this will appear in a later paragraph.

Subsequent investigators have had little to do except confirm the painstaking and elaborate description of Yamagiwa and Itchikawa. Here and there it has been possible to add an item or two, but on the whole our knowledge of the changes that occur in tarred epithelium has not been materially widened.

On account of the complexity of the material, the account of subsequent investigations will be discussed under two heads: those changes which have been observed in the epithelium, and those which run their course in the connective tissue.

Epithelium.—The fainter staining of the epithelium in early tar cancer, which Yamagiwa and Itchikawa noticed in hematoxylin preparations, has been studied by Fukuda [93] in sections impregnated by

Bielschowsky's method. He finds that whereas in normal epithelium only the deeper side of the basal cells is clear, in tarred animals the interpapillary epithelial processes become clearer with the progress of epithelial growth until finally, in early carcinoma, whole layers fail to take up the silver.

Menetrier, Peyron, and Surmont [182] have confirmed the epithelial lesions described by Yamagiwa and Itchikawa. They regard the almost complete absence of inflammatory reaction in the derma of the rabbit as a refutation of Ribbert's hypothesis, since it is evident that malignant growth must be initiated solely by the activity of the epithelium. Confirmation in all essentials has followed, also from Itchikawa and Baum [113] and Leroux and Simard,[158] as well as from Bittmann.[26]

Michail and Vancea,[187, 188] who studied the effect of tar on the eye of the rabbit, describe desquamation and later degeneration and hyperplasia of the epithelium. In the mouse, Busacca [46] mentioned hyperplasia of the connective tissue and epithelium of the cornea.

Deelman [67, 69] made wax reconstructions of tar carcinoma in the mouse, and carried out, also, a minute histologic study of its inception. He recognizes the following three phases:

1. Epithelial Growth (Papilloma, Hypertrophy of the Hair-Follicles and Sebaceous Glands). In this stage growth is always outward; and although there may be found areas of rapid proliferation, there is no invasion, and the epithelium does not assume an atypical appearance.

2. Atypical Epithelial Growth. Here the vigorously growing areas exhibit a distinctly atypical appearance, and may be said to be in the precancerous stage.

3. Malignant Epithelial Growth. In one or more areas of rapid growth, the atypical cells begin to invade.

The epithelial changes in the mouse have been described also by Dentici,[74, 75] Kashiwagi,[126, 126a] Roussy, Leroux, and Peyre,[233] Dreifuss and Bloch,[80] Bang,[3] E. Möller,[189] Lipschütz,[165] and Champy and Vasiliu.[55] Champy and Vasiliu followed the lesions almost from day to day, and counted the number of mitoses in 1,000 cells of the basal layer at various intervals. Their figures run as follows:

Normal skin	2–3
After 15 days' tarring	6
After 30 days' tarring	25
After 84 days' tarring	20
After 94 days' tarring	40
After 131 days' tarring	27
After 159 days' tarring	28
After 186 days' tarring	42

None of the authors just listed has described any material variation from the original observations of Yamagiwa and Itchikawa in the rabbit, except that there has been some discussion of the part taken by the hair-follicle. After reviewing the evidence, Yamagiwa [271] has come to the conclusion that there is a difference between the mouse and the rabbit in this respect; in the former species the superficial epithelium reacts more vigorously, whereas in the latter it is the hair follicle that is more responsive.

In an attempt to throw some light on the etiology of tar carcinoma, Itchikawa and Baum [115] investigated the lesions produced in two insusceptible species—the rat and the guinea-pig. In the rat they found a mild transient reaction in the epithelium and the derma, and a later atrophy of superficial epithelium, hair follicles, sebaceous glands and connective tissue. While there was some slight temporary reaction on the part of the blood vessels, this was soon followed by a phase in which all vascular reaction was totally lacking. The connective tissue was infiltrated with lymphocytes, polymorphonuclear leukocytes and a large number of mastocytes.

Comparing these lesions with those characteristic of the tarred rabbit, the authors concluded that the most striking difference was the absence of any lasting vascular reaction and the consequent atrophy of the skin.

Somewhat similar changes had been described in the rat by Paszkiewicz,[212] who mentioned atrophy of the hair follicles and sebaceous glands, effacement of the papillae, intense keratinization and increase in the connective tissue of the corium with hyaline degeneration and disappearance of the elastic fibers.

In the guinea-pig, Itchikawa and Baum [115] noted the same changes which they had seen in the rat, with the exception that cellular infiltration was not a prominent feature. As the lesions were similar in these two insusceptible species, they concluded that exemption from tar cancer was probably due to some common factor, and suggested that this might be the absence of vascular reaction and the atrophy which necessarily followed.

Besides morphologic studies on the epithelium, microchemical investigations have been carried out. Thus Waterman [267] finds that while under normal conditions potassium is in the epithelium and calcium in the corium, after tar painting potassium appears in the connective tissue and calcium in the epithelium, so that the distinction between these two layers gradually becomes wiped out. The process continues until finally the calcium is in the epithelium and the potassium in the connective tissue.

Connective Tissue.—The connective tissue has been investigated in great detail by a number of pathologists. The dilatation of the blood vessels described in the derma of the rabbit by Yamagiwa and Itchikawa

has been referred by Itchikawa and Baum [113, 114] to a paralysis of the peripheral nerves by some constituent of the tar. An intense connective tissue reaction was observed in connection with the regression of carcinomas.

In the mouse, Bierich [19, 21] has described a preliminary swelling of connective tissue fibrils and ground substance, a great increase in the quantity of elastin and an influx of mast cells. This stage lasts until epithelial invasion sets in, when there supervenes a second phase, characterized by regressive changes in the connective tissue in the course of which the swelling recedes, the mast cells disappear, and the tissue becomes less cellular and more vulnerable to invasion. The first stage, which appears to be of a defensive nature, could be duplicated by intensive roentgen-ray irradiation or by the administration of arsenic. Subsequent investigation [22, 23] showed that these alterations, which Bierich regarded as the sign of a colloid change, could be reproduced by soaking freshly removed skin in lactic acid, even to the increase of what had appeared to be elastic fibers.

A repetition of these experiments by Bierich and Rosenbohm,[25] in which they used buffered solutions of lactic acid with a p_H of from 2.6 to 8.4, led them to conclude that these fibers resulted from the hydrolysis of collagen.

This corresponds well with the view of Philippson,[216] who had already suggested that the increase in elastic fibers described by Bierich was only apparent, and due to a degeneration of connective tissue fibers which then took up the elastic stain.

Döderlein [78] found that the connective tissue of the mouse is the seat of a small round cell infiltration after about three weeks' tarring, and that later two zones could be clearly distinguished—one resembling granulation tissue and situated immediately beneath the epidermis, and just below this a zone of dense infiltration with small round cells.

Lipschütz [165] has described an edema of the mouse cutis combined with lively proliferation of its fibroblasts, among which were to be found large numbers of mast cells. There was a great dilatation of the capillaries and precapillaries, even in the underlying fat; indeed, these vessels might be so increased in caliber as to convey the impression that they had been injected, and Lipschütz had seen dilatation of the blood vessels even in the internal organs. In some cases observed by Bloch and Dreifuss [80] the capillaries were so enlarged and tortuous as to reproduce in miniature the corpus cavernosum. A relatively late change occurred in the subcutaneous fat, consisting of a lively proliferation of its fibroblasts and infiltration with lymphocytes, leukocytes, and a large number of mast cells. In some cases the fat was entirely replaced by this infiltrative process.

According to Borrel, Boez, and de Coulon,[39] swarms of branching, granular cells (mastocytes) collect at an early period in tarred mice, forming a veritable intradermic or subcutaneous nevus, homologous with the nevus of the human subject.

Dreifuss and Bloch[80] found the connective tissue of tarred mice loose, edematous and more cellular than normal even from the first, infiltrated with lymphocytes, plasma cells, polymorphonuclear leukocytes, and sometimes with mast cells. The most striking change in the vessels was their dilatation, which affected the larger vessels but above all the capillaries; these were increased in number, sometimes even to a point at which the base of a papilloma consisted wholly of tortuous and enlarged capillaries lying directly beneath the epithelium, with practically no connective tissue intervening.

Peyron[214] discussed the presence in the tarred mouse of numerous mastocytes and clasmatocytes, but pointed out that any hypothesis which would ascribe to these elements a special rôle, either in the genesis of cancer or in the defense of the organism against its inroads, was immediately contradicted by the fact that they are sparse in tar cancer of the rabbit. Thus, as Menetrier, Peyron, and Surmont[182, 183] have emphasized, it is essential that tar cancer be studied both in the mouse and in the rabbit.

The functional activity of the derma in tarred mice has been investigated by Foerster[92] with the aid of vital strains. Whereas normal mice show only occasional cells containing trypan blue, a few applications of tar are followed by an increase in the number of dye-laden cells. Their number increases through the papilloma stage and reaches a maximum with the development of carcinoma. They are regarded by Foerster as an expression of heightened metabolism which, however, seems to be confined to the irritated area, since the abdominal skin contains no more of them than that of untarred controls.

Struck by the similarity of tar carcinoma to the mixed tumor of the salivary glands, a neoplasm which probably develops from embryonal remnants and for the stroma of which Marchand has suggested the possibility of epithelial origin, Borst[41b] has raised the question whether the stroma of tar cancer may not be epithelial in nature.

(To be continued)

News and Notes

Death of Leishman.—Sir William Leishman, director-general of the medical service of the British army and an outstanding investigator of tropical diseases has died at the age of 60. He entered the army medical service on graduating in medicine at the University of Glasgow in 1886. After a time he devoted himself to microbiology, especially with respect to tropical diseases; in 1900, he became assistant professor of pathology in the army medical college at Netley and three years later professor of pathology in the army medical college at Millbank, London. He served on commissions for the investigation of tropical diseases in Africa. Second to Sir Almroth Wright, credit is given Leishman for placing antityphoid inoculation in the British Army on a sound and practical basis. His greatest work was in the group of protozoan diseases that came to bear his name — leishmaniosis. In 1900, he discovered the blood inhabiting organism first known as the Leishman-Donovan body but now called *Leishmania donovani,* in a soldier suffering from Indian kala-azar. Subsequently the Mediterranean form of kala-azar was recognized, and then so-called oriental sore and espundia or South American leishmaniosis were found to be caused by similar parasites, *Leishmania tropica* and *Leishmania americana.* Leishman's career and achievements contradict the current view that eminent pathologists are not good administrators and vice-versa. He continued in scientific pursuits after assuming duties of the highest post in the army medical service. His services in the Great War won him numerous honors, including the distinguished service medal of the U. S. Army.

French Physiologist Honored.—Charles Richet, professor of physiology (emeritus) in the University of Paris, and the discoverer of the phenomenon of anaphylaxis, has been presented with a bronze portrait bust and tributes from scientific societies throughout Europe.

University News, Promotions, Resignations, Appointments.—Dr. I. S. Falk has been promoted to the rank of associate professor in the department of hygiene and bacteriology of the University of Chicago.—Dr. Maxwell M. Wintrobe has been appointed to the Gordon Bell Memorial Fellowship of the College of Physicians and Surgeons at Manitoba; he will undertake research in connection with pernicious anemia and the leukemias.—Dr. Sedgwick Simons has been appointed instructor in clinical pathology in the Medical College of South Carolina, Charleston, to succeed Dr. Hugh E. Wyman, resigned.—Duke University, Durham, has established a fellowship for research in pernicious anemia, and Dr. Beaumont C. Cornell of Johns Hopkins Hospital, Baltimore, has been appointed to conduct the investigation.—Dr. Thomas A. Gonzales and Dr. Manuel E. Marten have been appointed deputy chief medical examiners in New York City. Both have been assistants to the medical examiners in the Manhattan and Brooklyn offices.—Dr. Garner Scullard, Pittsburgh, has been appointed pathologist at the Ohio Valley General Hospital, Wheeling.—Dr. Richard Douglas Passey, lecturer in pathology in the Welsh National School of Medicine, Cardiff, has been elected to the new chair of experimental pathology in the University of Leeds and will also be director of cancer research.—Dr. James Homer Wright, pathologist to the Massachusetts General Hospital

in Boston since 1896, has resigned and is now consulting pathologist.—Dr. Oscar Richardson, assistant pathologist to the Massachusetts General Hospital for twenty years, has resigned.—Dr. Tracy B. Mallory has been appointed head of the department of pathology in the Massachusetts General Hospital.—O. W. H. Mitchell, professor of bacteriology in the college of medicine of Syracuse University, has been given a year's leave of absence which will be spent traveling and studying in Europe.—Dr. Henry L. Jaffé has resigned as immunologist and bacteriologist to the Montefiore Hospital and accepted the directorship of the laboratories of the Hospital for Joint Diseases, New York.—Dr. Ernst Pribram, on leave of absence as professor of experimental pathology in the University of Vienna, has accepted appointments as pathologist to St. Elizabeth's Hospital, Chicago, and as assistant professor of pathology with part time duties in the University of Chicago (Rush Medical College).—Dr. M. Pellissier, Lille, professor of pathology, and interested especially in medicolegal problems, has died, aged 40.

Society News.—Dr. G. E. Fahr has been elected president of the Minnesota Pathological Society and Dr. E. T. Bell, secretary-treasurer.—Dr. Lloyd Arnold has been elected president of the Chicago Pathological Society and Dr. E. F. Hirsch reelected secretary. In 1925, Dr. George H. Weaver declined reelection as secretary of this society after having served as such continuously for thirty-one years.

Foot-and-Mouth Disease Commission Returns.—The commission consisting of Dr. Peter K. Olitsky, Rockefeller Institute for Medical Research, Jacob Traum, D.V.S., University of California, and Dr. Harry W. Schoening, U. S. Bureau of Animal Industry, which was sent to Europe by the Department of Agriculture to study foot-and-mouth disease, has returned after more than a year abroad. The commission established itself at the Institut d'hygiène, Strasbourg, and later at the Laboratoire national de recherches, Alfort, France, where experimental studies were made on the etiology of foot-and-mouth disease from its physical, chemical and biologic aspects. European investigators and the officials gave fullest cooperation in this work, and studies were made of control measures and research work in England, Denmark, Sweden, Holland, Belgium, Switzerland, Austria, Hungary, Italy, France and Germany.

Abstracts from Current Literature

Pathologic Physiology

HEART OUTPUT DURING PREGNANCY. H. J. STANDER, E. E. DUNCAN and W. E. SISSON, Am. J. Obst. & Gynec. **11**:44 (Jan.) 1926.

The authors have employed the principle of Fick, which later was elaborated by Zuntz, that a minute volume of blood is equal to the total oxygen used by an animal per minute divided by the difference in the oxygen per cubic centimeter of arterial and venous blood. The dogs selected were carefully trained to eliminate psychic errors. Arterial and venous blood were taken from the left and right side of the heart, respectively, and the oxygen content determined by the Van Slyke method. For the expired air the Haldane gas analysis apparatus was employed. Six dogs were used, four of which were nonpregnant, and two were studied in their nonpregnant, pregnant and puerperal state. It was shown that the heart output was definitely increased, the maximum being reached toward the end of the gravid period and diminishing gradually to its normal status during puerperium. An increased cardiac output during the estrus cycle was demonstrated, similar to that of pregnancy but of a slighter degree.

ALFRED J. KOBAK.

AN INVESTIGATION INTO THE CAUSATION OF THE ONSET OF LABOR BY PARABIOSIS DURING PREGNANCY. I. KROSS, Am. J. Obst. & Gynec. **11**:64 (Jan.) 1926.

Kross repeated the work that Sauerbruch and Heyde did in 1910, employing 11 pairs of rats united in parabiosis. In the animals thus united one member of each pair was in a further state of pregnancy than the other. Two pairs died, and of the remaining nine, in only one pair did the parturition of one induce labor of the other animal before its term. In view of the normal periods of gestation in eight pairs of these artificially produced "Siamese Twins," Kross does not accept the theory of anaphylactic reaction suggested by Sauerbruch and Heyde.

ALFRED J. KOBAK.

THE RELATION OF THE FILTERABILITY OF DYES TO THEIR EXCRETION AND BEHAVIOR IN THE ANIMAL BODY. ARTHUR GROLLMAN, Am. J. Physiol. **75**:287 (Jan.) 1926.

As a result of the study of dye concentrations in the urine and blood of frogs that have received injections, the author finds that the ability of the kidney to eliminate dyes introduced into the blood stream is intimately related to, and in large part determined by, their filtrability. In connection with in vitro experiments, he calls attention to the necessity of continuing ultrafiltration until true equilibrium is established between membrane and fluid. Also to the fact that filtrability may alter in Ringer's solution or blood serum from its capacity in this respect in aqueous solution.

H. E. EGGERS.

THE EFFECT OF A PROTEIN-FREE ACID-ALCOHOL EXTRACT OF THE PARATHYROID GLANDS UPON THE CALCIUM CONTENT OF THE BLOOD AND THE ELECTRICAL IRRITABILITY OF THE NERVES OF PARATHYROIDECTOMIZED AND NORMAL ANIMALS. LOUIS BERMAN, Am. J. Physiol. **75**:358 (Jan.) 1926.

The experiments of the author show that on injection into dogs of an extract of parathyroid with acid-alcohol at room temperature, there follows an increase of blood calcium and a decrease of electrical excitability of nerves. In the case of parathyroidectomized animals in tetany the symptoms are relieved, blood calcium is raised to approximately its normal level and the threshold of electrical excitability is raised. The last appears to be essentially an effect of the calcium mobilization.

H. E. EGGERS.

THE EFFECT OF BLEEDING UPON THE SERUM CALCIUM OF PARATHYROIDECTOMIZED DOGS. W. W. SWINGLE and W. F. WENNER, Am. J. Physiol. **75**:372 (Jan.) 1926.

Following considerable withdrawal of blood in dogs suffering with tetania parathyropriva, there occurs a prompt relief of symptoms along with a marked rise of serum calcium. This again decreases after ten or twelve hours, whereon tetany again appears.

H. E. EGGERS.

STUDIES ON THE CONTROL OF BLOOD PRESSURE WITH HEPATIC EXTRACT. A. A. JAMES, N. B. LAUGHTON and A. BRUCE MACALLUM, Am. J. Physiol. **75**:392 (Jan.) 1926.

The results reported are: effectual reduction of hypertension induced in rabbits by certain pressor substances (epinephrine, pituitary extract, etc.); reduction to a low level of blood pressure in normal rabbits, this persisting for long periods; death from too great reduction of blood pressure consequent on large doses.

The effects obtained differ materially from those obtained with histamine or choline; chemical tests for these substances are negative. The active substance is ether-soluble, is obtainable by the writers' method from fresh liver tissue only, and does not give the biuret reaction.

H. E. EGGERS.

LIVER FUNCTION TESTS. A COMPARATIVE STUDY OF FIVE METHODS IN 100 CLINICAL CASES. SAMUEL S. BERGER, MILTON B. COHEN and J. J. SELMAN, J. A. M. A. **86**:1114 (April 10) 1926.

The tests used were van den Bergh's, Widal's hemoclastic crises test, Rosenthal's, and examination of the urine for bile salts and for urobilin and urobilinogen. The authors' conclusions follow:

1. It is important to bear in mind that these tests represent different functions of the liver. Any one or more or all of these functions may become impaired. Again, one or more of these functions may escape injury. Therefore the various tests do not give parallel results. When we attempted to separate clinical cases into groups of liver disease or no liver disease by means of any one of these tests, unsupported by other clinical evidence, we were unable to do so.

2. When all the tests were positive, we were dealing with liver disease, clinically of the most severe type; namely, toxic jaundice.

3. When all tests were positive except one, namely, four positive and one negative, clinical liver disease was present, usually of a chronic type, such as that seen in Banti's disease or pernicious anemia and cirrhoses.

4. In every case in which all the tests were positive except the Widal, there was obstructive jaundice due to tumor. This finding is of great value in differential diagnosis.

5. When only three tests were positive it was impossible to correlate the findings with the clinical picture, as there were many cases in which liver disease was suspected which did not give positive reactions to more than one or two tests and, conversely, there were many cases in which liver disease was unsuspected which gave as many positive results.

6. At present they are of use chiefly in the differential diagnosis and in following the progress of a given case. The greatest amount of information can be gained by doing all the tests simultaneously and repeating them often.

AUTHORS' SUMMARY.

THE RELATION OF ALBUMINURIA TO PROTEIN REQUIREMENT IN NEPHRITIS. J. P. PETERS and H. A. BULGER, Arch. Int. Med. **37**:153 (Feb.) 1926.

A study of the nitrogen metabolism of certain patients with acute and chronic parenchymatous nephritis has been made in an attempt to determine to what extent protein lost as albumin in the urine can be replaced by food protein. The total urinary nitrogen of these patients is not a satisfactory measure of nitrogen catabolism. The nitrogen catabolism can be estimated only from the urinary nonprotein nitrogen after proper allowance has been made for changes in blood and tissue nonprotein nitrogen and variations of body weight due to diuresis or accumulation of edema. By the administration of large amounts of carbohydrate and fat it has proved possible to reduce the protein catabolism to from 0.5 to 0.7 Gm. per kilogram a day. If enough protein is given to cover the nitrogen catabolism plus an additional amount equivalent to that lost as albumin in the urine, nitrogen wastage may be prevented. Most of these patients when they enter the hospital show evidences of previous protein deficiency. If they are given more than enough protein to replace the amount lost in the urine, they will store the excess within certain limits, thus repairing the effects of previous nitrogen wastage. This protein wastage may be due partly to early dietary mismanagement. The loss of protein as albumin in the urine is certainly a serious contributory cause. It also seems probable that the disease itself, in its more acute stages at least, is characterized by a higher protein metabolism than normal. This is suggested by the results of these studies. Abnormally high blood nonprotein nitrogen has been observed only when the nitrogen catabolism was relatively high and has usually returned to the normal level as the clinical condition of the patient improved and the nitrogen catabolism diminished.

S. A. LEVINSON.

GASTRIC SECRETION IN EXPERIMENTAL BERIBERI IN THE DOG. M. B. FARNUM, Arch. Int. Med. **37**:212 (Feb.) 1926.

Feeding a beriberi producing diet to a dog gradually decreases the total volume as well as the free and the total acidity of gastric secretion. The gastric secretory mechanism of the beriberi dog is not only less responsive

to the partaking of food, but it is also appreciably more refractive toward histamine hydrochloride injection. The gastric response after injection of gastrin is modified only slightly if at all. The harmful effect of alkali in the autoclaving process is not due to racemization of the proteins but to destruction of the antineuritic vitamin. The antineuritic vitamin is not a gastric secretagogue.

S. A. Levinson.

Changes in Respiration, in Circulation and in the Coagulation Time of Blood Produced by Sodium Citrate Injections. M. Joannides, Arch. Int. Med. **37**:248 (Feb.) 1926.

These studies on man and on the dog show that the injections of sodium citrate do not usually increase the coagulability of the blood. Intramuscular injections are less dangerous than intravenous injections. A 3 per cent solution of sodium citrate, which is isotonic with blood, has been fairly well tolerated by a patient who received from 300 to 450 cc. intravenously. This practice, however, should be discouraged. Intravenous injections of great concentrations of sodium citrate are dangerous because they cause immediate respiratory and circulatory collapse. Calcium chloride given promptly during the height of toxic symptoms may save the animal from death but calcium chloride itself is a dangerous drug if injected intravenously in large doses. The toxic symptoms must in some way be associated with changes that concern the coagulation of the blood, because the latter becomes incoagulable under these conditions. As soon as the toxic symptoms subside, the coagulation time of the blood becomes normal. Shallow, slow breathing, a rapid and later slow pulse rate, convulsions, at times salivation and incontinence of urine, with a drop in the systolic pressure and increased coagulation time, mark the presence of toxic effects of sodium citrate. Small, repeated hemorrhages apparently have no effect on the coagulation time. On the other hand, when larger amounts of blood are removed, they cause an increased coagulability of the blood. Citrated blood transfusion under such conditions increases the coagulability of the blood still more. There is no apparent difference in the coagulation time of the arterial and of the venous blood. Injections of 0.2 per cent sodium citrate solution in a 0.75 per cent sodium chloride solution invariably decrease the coagulation time of the blood and cause no toxic symptoms if the citrate dose is within the subtoxic limits. Sodium citrate apparently disappears from the blood stream promptly, since the toxic symptoms produced by the salt disappear promptly. Sodium citrate used in amounts corresponding to those for blood transfusion is a useful drug. In larger amounts or concentrations it is a dangerous drug.

S. A. Levinson.

Evidence of Nervous Control of Leukocytic Activity by the Involuntary Nervous System. E. F. Mueller, Arch. Int. Med. **37**:268 (Feb.) 1926.

The number of leukocytes found in any vascular region of the body depends on the balance of the involuntary nervous system in this particular region. The leukocytes will be evenly distributed as long as the involuntary nervous system, vasodilators and vasoconstrictors are in normal balance. The uniform distribution changes immediately if there is an overbalance in either direction. Such an overbalance never takes place in the entire body. If large areas are involved, peripheric and splanchnic regions balance each other. An overbalance of the sympathetic, vasoconstricting portion causes local leukopenia, while

overbalance of the parasympathetic, vasodilating part causes leukocytosis. Nervous action is always the primary cause of leukocytic activity as far as accumulation and decrease are concerned, even though the leukocytes are in no way directly connected with either the walls of the vessels or the vasocontrolling nerves of the involuntary nervous system. This knowledge may furnish a new link connecting immunity and body resistance (of which the leukocytic activity is only one part) with the teachings of pathology and physiology in the human economy.

S. A. LEVINSON.

INTRADERMAL SALT SOLUTION TEST IN CARDIAC DISEASE IN CHILDREN. H. C. OLMSTED, Arch. Int. Med. **37**:281 (Feb.) 1926.

In a group of forty-seven children with rheumatic heart disease, or allied conditions, it was found that: The disappearance time of intradermally injected salt solution was normal (above fifty minutes) in well compensated nontoxic cardiac disease, acute rheumatic fever and chorea. In cardiac decompensation with edema, shortening of disappearance time was limited to edematous and preedematous regions, and was noted earliest in the dependent parts of the body. Decrease in disappearance time did not precede other clinical signs of impending decompensation with sufficient constancy to make the test of much value in predicting a break in compensation. In certain severely ill patients who had cardiac disease without decompensation there was a marked and nearly equal decrease in disappearance time in arm and leg. The intradermal salt solution test was of value in this generalized or toxic group in rendering an unfavorable prognosis. The disappearance time picture characteristic of the toxic group was altered in the direction of that characteristic of the decompensation group, if cardiac decompensation developed. In two patients tested after death, the disappearance time in edematous parts was practically as short as during life, which demonstrated that, under these conditions, circulation of the blood was not necessary in the phenomenon of disappearance of intradermally injected salt solution.

S. A. LEVINSON.

THE INFLUENCE OF MAGNESIUM SULPHATE ON THE EXPULSION OF BILE FROM THE GALLBLADDER. W. H. GANTT and G. VON VOLBORTH, J. Lab. & Clin. Med. **11**:542, 1926.

Using a special device for maintaining normal pressure in the bile valves, the authors show absolutely no influence of magnesium sulphate on the expulsion of bile into the duodenum.

S. A. LEVINSON.

JAUNDICE FOLLOWING PULMONARY INFARCTION IN PATIENTS WITH MYOCARDIAL INSUFFICIENCY—A CLINICAL STUDY. C. KEEFER and W. RESNIK, J. Clin. Investigation **2**:375, 1926.

Jaundice occurring in myocardial insufficiency of long standing, particularly in patients with mitral and tricuspid disease and auricular fibrillation, is a well recognized condition. The authors point out the sudden appearance or marked increase in jaundice which occurs after pulmonary infarction in patients suffering from myocardial insufficiency. Ten cases of heart failure were studied: five syphilitic, four rheumatic and one arteriosclerotic. Four of these gave a history of pulmonary infarction, and six of them had slight jaundice on admission. The clinical picture was almost similar in all of them. All were moderately advanced cases. Slight improvement took place under treatment.

Suddenly the clinical course changed, dependent on the development of pulmonary infarction. Dyspnea became more intense, cyanosis deepened, myocardial failure increased and progressed rapidly. Usually, on the second or third day after the occurrence of infarction, jaundice suddenly made its appearance, or it became markedly intensified if it was present previously. At the same time bilirubin and urobilin were found in the urine. Hemorrhagic phenomena occasionally developed. These symptoms increased, advanced steadily, and within a relatively short time death ensued in all the reported cases. There are cases, however, in which pulmonary infarction produces lesser degrees of jaundice detectable only in the van den Bergh test in which the clinical course is not so stormy and recovery frequently occurs.

M. L. PARKER.

BLOOD CHOLESTEROL AND BLOOD PRESSURE. G. RICHARD and J. ROESCH, Bull. Acad. de méd., Paris **95**:363, 1926.

There was no parallelism between the degree of arterial hypertension and the amount of blood cholesterol in eighty persons with high blood pressure, and there was no connection between the content of urea and of cholesterol in the blood, although hypercholesteremia was frequently present in chronic interstitial nephritis disease. A considerable amount of nonprotein nitrogen may be accompanied by only slight hypercholesteremia. All the patients with chronic and severe headache presented a notable cholesteremia, with increased nonprotein nitrogen and rather low blood pressure.

HYPERCHOLESTEREMIA AND NAPHTHALENE CATARACT. D. MICHAIL and P. VANCEA, Compt. rend. Soc. de biol. **94**:291, 1926.

Rabbits were fed with a mixture of glycerol and naphthalene, and after from twelve to twenty days retinal lesions followed by cataract developed. In the meantime, the amount of cholesterol in the blood had doubled or tripled. In senile cataract there is hypercholesteremia but not in congenital and juvenile cataract.

VIABILITY OF TRANSFUSED ERYTHROCYTES. H. WILDEGANS, Arch. f. klin. Chir. **139**:135, 1926.

After direct transfusion into an anemic patient of blood from a patient with polycythemia, the transfused corpuscles could be recognized clearly in the blood of the recipient for two weeks.

HEREDITARY PSEUDOHEMOPHILIA. E. A. VON WILLEBRAND, Finska läk.-sällsk. handl. **68**:87, 1926.

Willebrand describes a family with not less than twenty-three bleeders among the sixty-six members in four generations. The coagulation time was normal, but the bleeding time was extremely long in the grave cases, and the Rumpel-Leede phenomenon — minute subcutaneous hemorrhages below rubber bandage to upper arm for ten minutes — was pronounced. The clinical picture resembled that of Weil's disease although certain features differed, suggesting some functional disturbance in the thrombocytes plus a general lesion of the vessel walls. The thrombocytes numbered from 55,000 to 450,000. The eosinophils ranged from 1 to 14 per cent, the lymphocytes from 25.3 to 61.3 per cent, and the

neutrophils from 35 to 65.4 per cent. It is questioned whether this may not represent a new nosologic type of hemorrhagic diathesis. The graver forms were in women and proved fatal in five. None of the male members died from this cause. Transmission seemed to be in the female line. Nineteen cases on record are summarized.

On the Excretion of Phenolsulphonphthalein from Normal Kidneys. Also a Study of the Technic of the Phenolsulphonphthalein Test. Shichtaro Sugimura and Tetsutaro Aomura, Tohoku J. Exper. Med. **7**:125, 1926.

Following subcutaneous, intragluteal and intravenous injections of phenolsulphonphthalein preceded by 300 to 500 cc. of water by mouth, and after intravenous injections not preceded by a water intake, nephelometer determinations of the time of the first appearance of the dye in the urine, and of the amounts excreted during each half of the first hour and each of the three succeeding hours in twenty-four persons with healthy kidneys, varied within wide limits according to the preparation used, the technic of the injection and the colorimetry. The peak of the excretion after intravenous injection preceded by a water intake came within the first half hour with a rapid decline later, and the total excretion, 76.6 per cent during the four hours, exceeded that from any other method of administration.

Concerning the Sympathetic Sensitizing Action of the Serum of Hypertension. Sakari Tashiro, Tohoku J. Exper. Med. **7**:144, 1926.

In testing the thresholds for electrical stimuli before and after treating the cervical sympathetics of cats with serums from normal and nephritic persons, an increased irritability was found chiefly with the use of serum from diffuse glomerular nephritis with increased blood pressure, from secondary contracted kidney and malignant hypertension (genuine contracted kidney). This sensitizing action was lacking in serums from essential or mild hypertension and chronic glomerular nephritis without increased blood pressure.

Pathologic Anatomy

The Reticulum of the Lung: III. Its Rôle in the Healing of Miliary Tubercles. W. S. Miller, Am. Rev. Tuberc. **13**:360, 1926.

Associated with the development of a miliary tubercle in the lung, there is an increase in reticulum; the reticulum being "a precollagenous type of tissue, which by further development is converted into collagenous (fibrous) tissue." A careful analysis of tubercles at different stages of development, all taken from one lung, indicate that as tubercle bacilli increase in number and polymorphonuclear leukocytes invade the tubercle, caseation is progressing. Disappearance of leukocytes, immigration of monocytic cells and an increase in reticulum and fibrous tissue go hand in hand. The conversion of reticulum into collagenous fibers is therefore a significant part in the healing process, and "whatever tends to promote the formation of reticulum and its conversion into collagenous tissue, tends to promote the healing of a tubercle."

Max Pinner.

A Microscopic Study of the Living Kidney After the Injection of Dyes. J. G. Edwards, Am. J. Physiol. **75**:330 (Jan.) 1926.

The dyes studied come under three categories: (1) those readily eliminated, of the type of phenolsulphonphthalein, in which coloration of the glomeruli appears immediately after injection, followed by a diffuse coloration with the appearance of the dye in the tubular lumina after from twenty-five to thirty-five minutes; (2) those less readily eliminated, as trypan blue, in which the excretion appears to be largely tubular in character, with appearance of the dye in the epithelium of the proximal convoluted tubules; (3) dyes not eliminated through the kidney, as gentian violet. Here no renal staining is seen.

H. E. Eggers.

Regeneration of Pancreatic Tissue from the Transplanted Pancreatic Duct in the Dog. J. W. Shaw and E. O. Latimer, Am. J. Physiol. **76**:49 (March) 1926.

Segments of pancreatic duct, completely freed from the glandular tissue, were inserted under the intestinal serosa, a complete pancreatectomy also being performed. The dogs were kept alive with insulin, and the animals were examined after two months or longer. In all animals in which this time was allowed, there was found evidence of regeneration of ductlets, with some indication of acini and islet formation. In the cases in which the duct did not establish communication with the surface, there was an accumulation of secretion, with probable retardation of regeneration.

H. E. Eggers.

Necrosis of the Corpus Luteum of Pregnancy. Dorsey Brannan and Mortimer Cohen, Surg. Gynec. Obst. **42**:228, 1926.

Necrosis of the corpus luteum may occur in pernicious vomiting of pregnancy.

Necrosis of the corpus luteum in pernicious vomiting of pregnancy probably has the same significance as has necrosis of the liver and kidneys in this disease.

Author's Summary.

Intrahepatic Cholelithiasis. E. Stan Judd and V. G. Burden, Surg. Gynec. Obst. **42**:322 (March) 1926.

A case is reported in which many large intrahepatic calculi were found in a liver which was grossly normal. The patient was operated on eleven years previously, at which time cholecystectomy was performed, and many stones were removed from the extrahepatic ducts. The present condition existed without the occurrence of jaundice or any clinical evidence of hepatic insufficiency, the calculi being found at necropsy following death from intestinal obstruction. Intrahepatic stones occur rarely. Their practical significance from a surgical standpoint is not of much consequence, but their occurrence must be kept in mind as an occasional cause for recurrence of symptoms after operations on gallbladder and ducts. The frequency of stones in the gallbladder and common duct, their less common occurrence in the hepatic duct and their almost complete absence from the ducts within the liver, have led to the assumption that all stones form in the gallbladder. The small bits of gravel which sometimes form in the liver probably pass through the ducts without difficulty.

M. L. Parker.

FURTHER OBSERVATIONS ON AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF THYROID GLAND IN THE GUINEA-PIG. LEO LOEB, Am. J. Path. **2**:99, 1926.

After autotransplantation a gradual elimination takes place of those factors which distinguish an early transplant from the normal organ. The mutual equilibrium between adjoining tissues is reestablished owing to the action of autosubstances given off by acinus cells and acting on the connective tissue cells, blood vessels and lymphocytes.

The reactions following homeotransplantation of thyroid and parathyroid depend mainly on the aggressive activity of the lymphocytes and connective tissue cells of the host to which a certain type of vascularization corresponds. Variations in the intensity of this reaction are essentially graded in accordance with the genetic relationship of donor and host, but there are some indications that nongenetic variable factors may to a certain extent modify the intensity of the reaction.

LEO LOEB.

AUTOTRANSPLANTATION AND HOMOIOTRANSPLANTATION OF CARTILAGE IN THE GUINEA-PIG. LEO LOEB, Am. J. Path. **2**:111, 1926.

The reactions against autotrasplants as well as homeotransplants of cartilage are in principle the same as those against other tissues. After homeotransplantation, lymphocytic as well as connective tissue reactions take place. However, cartilage shows a greater power of survival after homeotrasplantation than the majority of other tissues. This is due to three factors: (1) a diminution in the intensity of the homeotoxic action, (2) a greater resistance of the cartilage toward the instruments of attack on the part of the host, namely, the lymphocytes and connective tissue cells and (3) a decrease in the intensity of the lymphocytic reaction which takes place in the course of time. The individuality differential is developed in cartilage as well as in other tissues; but it manifests itself with less vigor in the cartilage, owing perhaps to the relatively low metabolism of cartilage.

LEO LOEB.

SECONDARY GLIOMATOSIS OF THE LEPTOMENINGES. DORSEY BRANNAN, Am. J. Path. **2**:123, 1926.

Diffuse secondary gliomatosis of the cerebrospinal leptomeninges is a distinct pathologic entity.

AUTHOR'S SUMMARY.

ORGAN WEIGHTS OF NORMAL RABBITS. SECOND PAPER. WADE H. BROWN, LOUISE PEARCE and CHESTER M. VANALLEN, J. Exper. Med. **46**:733, 1926.

The results of a second series of organ weight determinations on normal rabbits are reported, and the values obtained are compared with those for the first series. Figures are also given which represent the results obtained by combining the two series of animals or from weight determinations made on 645 apparently normal rabbits selected from stocks used for various experimental purposes.

AUTHORS' SUMMARY.

THE PRESENCE OF DESQUAMATED ENDOTHELIAL CELLS. THE SO-CALLED CLASMATOCYTES, IN NORMAL MAMMALIAN BLOOD. FLORENCE R. SABIN and CHARLES A. DOAN, J. Exper. Med. **46**:823, 1926.

There is a practically constant desquamation of endothelial cells into the circulating blood in rabbits and man. These endothelial cells represent the entire range in size of endothelium from very small cells possibly from capil-

laries, up to the type of the large Kupffer cell. These desquamated cells can be discriminated from monocytes.

There are common morphologic and functional characteristics among the following cells: the clasmatocytes from the specific endothelia, such as the cells from the sinuses of the liver, spleen and bone marrow; the clasmatocytes of the diffuse connective tissues, and desquamated endothelial cells occurring in normal and pathologic blood.

There are four strains of white cells in the normal circulating human and rabbit blood granulocytes, lymphocytes, monocytes and endothelial phagocytes.

AUTHORS' SUMMARY.

NORMAL AND PATHOLOGICAL FRAGMENTATION OF RED BLOOD CELLS; THE PHAGOCYTOSIS OF THESE FRAGMENTS BY DESQUAMATED ENDOTHELIAL CELLS OF THE BLOOD STREAM; THE CORRELATION OF THE PEROXIDASE REACTION WITH PHAGOCYTOSIS IN MONONUCLEAR CELLS. CHARLES A. DOAN and FLORENCE R. SABIN, J. Exper. Med. **46**:839, 1926.

There is constantly some breaking down of red cells in the circulation by fragmentation. The fragments of red cells as well as whole red cells are enveloped and destroyed by clasmatocytes or endothelial phagocytes.

When there is an increase in fragmentation in abnormal or pathologic states, desquamated endothelial cells of the blood stream, as well as the clasmatocytes of the tissues, increase proportionately and take in these fragments. These cells are to be distinguished from eosinophilic leukocytes by the nature of their granules, by the type of motility of the cells and by a negative peroxidase test.

The desquamated endothelial cells, clasmatocytes, in the circulating blood are positive to the peroxidase test only when they have taken in positive material.

The monocytes show marked variations of the oxidase reaction in different species and to different technics. With the Sato and Sekiya technic most monocytes of human blood are positive, while most of them in rabbit blood are negative, but both positive and negative reactions are found in both human and rabbit blood.

AUTHORS' SUMMARY.

PALPABLE ARTERIAL COATS IN CHILDREN. A. E. VIPOND, Brit. J. Child. Dis. **23**:43, 1926.

Vipond found that a number of children, especially among the undersized, had thickened arterial walls, so that the vessels could be palpated and felt to roll under the fingers. He says that it is a congenital condition affecting the entire arterial system, not associated with syphilis, and distinctly familial, demonstrable always in one of the parents as well as in the children. There are no symptoms and no changes in the heart or kidneys; the blood pressure is normal or slightly increased.

B. R. LOVETT.

CONTRIBUTION TO THE MICROSCOPIC CHANGES OF TUBERCULOSIS IN GUINEA-PIGS. W. PAGEL, Beitr. z. klin. d. Tuberk. **63**:160, 1926.

Attention is drawn to the histologic changes in the blood vessels in tuberculosis. Not infrequently small nodules on the intima are observed, particularly in animals that had received cholesterol and vital dyes. These nodules are composed of proliferating endothelial cells, of cells with spindle shaped nuclei

lying in a homogenous substance or of connective tissue with many nuclei. These granulomas never contain tubercle bacilli. The author believes that these observations are the manifestation of a specific endothelial reaction to the tuberculous infection. Tuberculous cavities in guinea-pigs should never be diagnosed macroscopically, since emphysematous blebs or nontuberculous bronchiectases may simulate cavities.

MAX PINNER.

PRIMARY TUBERCULOSIS OF THE MIDDLE EAR. A. GHON and H. KUDLICH, Ztschr. f. Hals-, Nasen- u. Ohrenh. **14**:77, 1926.

The case of a male infant is reported, in whom the primary tuberculous infection was located either in the middle ear or in the pharynx; the regional lymph glands were caseated. The further propagation followed apparently the following channels: (1) lymphogenous, to the portal of entrance into the venous system at the angle between jugular and anonymous veins; (2) hematogenous, from the portal of entrance to the lungs, where miliary, perivascular tubercles were found without involvement of the regional lymph glands, which permits the exclusion of a primary pulmonary infection; (3) the arterial system to the spleen and possibly to the liver; (4) canalicular, through the intestinal tract to the lower ileum, where tuberculous ulcers were located. The child had been operated on for tuberculosis of the middle ear; this is the reason why no definite decision could be made whether the first infection was in the middle ear or in the pharyngeal tonsil, since both organs share in the regional lymph glands.

MAX PINNER.

LYMPHOGRANULOMATOSIS OF THE GASTRO-INTESTINAL TRACT. H. DROPE, Virchows Arch. f. path. Anat. **259**:147, 1926.

Drope reports a case of Hodgkin's disease in which the primary localization was in the stomach, the process remaining limited to the gastro-intestinal tract and mesentery.

O. T. SCHULTZ.

STAINING OF GRANULES OF CHIEF CELLS OF GASTRIC MUCOSA. H. HAMPERL, Virchows Arch. f. path. Anat. **259**:179, 1926.

Hamperl describes a method for staining the granules of the chief cells of the gastric gland tubules. The essentials of the method are fixation in an alcoholic potassium acetate formalin mixture (formalin, 33 cm.; 80 per cent alcohol, 66 cm.; potassium acetate, 3 to 6 Gm.); embedding in paraffin; staining of the sections in dilute methyl violet, followed by differentiation in absolute alcohol, clearing in xylol and mounting in balsam.

O. T. SCHULTZ.

RIB IMPRESSIONS ON THE LUNGS OF INFANTS. T. KONSCHEGG, Virchows Arch. f. path. Anat. **259**:225, 1926.

Furrows made by the ribs on the surface of the paravertebral portions of the lungs of infants are frequently seen. They may number one to ten. Factors in their formation are emaciation and increased intra-abdominal tension, the latter leading to decreased diaphragmatic breathing, with greater distention of the upper portions of the lungs through the action of the accessory muscles of respiration.

O. T. SCHULTZ.

STATUS MARMORATUS. A. MEYER, Ztschr. f. d. ges. Neurol. u. Psychiat. **100**:529, 1926.

The theories as to the pathogenesis of this peculiar marble-like appearance of the striate body in children is discussed. C. Vogt considers it a heredo-degeneration and shows that the fibromyelin plaques may occur in other congenital malformations in the cortex. Scholz believes that an exogenous toxin probably that of scarlet acts on the striatum when it is fully developed and causes glial scars and myelin hypertrophy. He terms the process infantile striatal sclerosis. The author's own case revealed diffuse endarteritis of the cortical arterioles with fatty changes in the endothelium. There were small atrophic areas about the vessels which were invaded by fine glial fibers. All of the basal ganglions were thus affected, the putamen most severely. The internal capsule was partly sclerotic. Meyer believes his case resulted from the vascular lesion and not from a developmental defect.

ROY GRINKER.

THE PATHOLOGY OF SUBARACHNOID HEMORRHAGE. M. MARGOLIA, Ztschr. f. d. ges. Neurol. u. Psychiat. **100**:616, 1926.

A fatal case of spontaneous hemorrhage was studied. The basal subarachnoid space about the pons and medulla was filled with blood, and in the substance of the pons were many small hemorrhages. There were in the meninges small macrophages without contents and many large mulberry cells containing from 10 to 12 red cells. There was an arteriosclerosis of the small pial vessels with some hyalinization. The hemorrhages were ascribed to diapedesis through the damaged vessels.

ROY GRINKER.

FAT IN THE CENTRAL NERVOUS SYSTEM OF THE NEW-BORN. P. SCHWARTZ, Ztschr. f. d. ges. Neurol. u. Psychiat. **100**:713, 1926.

The author discusses the conception that lipoid material in the glia cells of infants is concerned with the physiologic development of myelin. He concludes that all the brains of dead new-born are pathologic. There are small hemorrhages, glia proliferations or axis cylinder changes which may be due to intra-uterine infections, asphyxia or trauma at birth. The lipoids in the glia are definitely related to the pathologic process and are not concerned with the building up of the myelin.

ROY GRINKER.

CONTRIBUTION TO THE STUDY OF PETRIFIED FETUSES (CASE OF LITHOKELYPHOPEDION). ELLA FASTENAU, Frankfurt. Ztschr. f. Path. **33**:200, 1925.

At the necropsy of a woman, aged 75, who died of apoplexy and hypostatic pneumonia, a tumor was found in the omentum — 20 cm. long, stone hard, with a calcified outer shell, and containing a fetus probably 30 cm. in full length, in which the skull, ribs, vertebrae, pelvis and femur, as well as the brain, lungs, intestine, stomach and liver, were recognizable. A strand of what might have been obliterated blood vessels extended from the concave side of the fetus to a walnut sized calcified body in the fimbriated end of the right tube. Microscopically little nuclear staining could be obtained. The fetal membranes were calcified and coalesced with the skin, which, with the subcutaneous tissue and upper muscle layers, was also calcified. This fetus must have been carried for at least thirty years, and apparently without ill effects to the woman.

This must be classified as a lithokelyphopedion, according to Küchenmeister (1881) who grouped the forty-eight cases from the literature into lithokelyphos, lithokelyphopedion, and true lithopedion indicating respectively the calcification of membranes only, of both membranes and fetus with coalescence and of the fetus alone. To the statistics of R. Freund, up to 1903, are added summaries of reports since that time, including the author's own case described here.

E. N. HALL.

CONCERNING THE APPEARANCE OF FAT-GIANT CELLS. ELSE PETRI, Centralbl. f. allg. Pathol. u. path. Anat. **37**:1, 1926.

In a patient dying of carcinoma of the tongue, Petri found atrophic hemp seed sized masses containing lymphoid, plasma and eosinophilic cells in the prevertebral tissues. Accompanying these were giant cells containing vacuolated protoplasm, and on a basis of morphology these were called fat-giant cells, but no fat stains were made. A stimulating effect of the carcinoma is the author's theory as to the occurrence of fat-giant cells, and he cites experimental evidence of the occurrence of such cells after hunger, hibernation and transplantation of fat tissue.

GEORGE J. RUKSTINAT.

DIFFUSELY PIGMENTED GLIOMA OF THE LEFT CEREBRAL HEMISPHERE. KARL VON WOLFF, Centralbl. f. allg. Pathol. u. path. Anat. **37**:5, 1926.

A tumor 8 by 6 by 5 cm., its upper level at the corpus callosum, occupied parts of the left temporal and occipital lobes and caused a forward displacement of the left optic thalamus, compression of the third ventricle and a backward and lateral displacement of the cerebellum and corpora quadrigemina. Histologically the mass contained a ground substance of glia fibers supporting a pigmented glia syncytium in which the nuclei had marked variations in the size of their chromatin granules. The author agrees with E. J. Kraus that the pigment was a fat-albumin compound derived from degenerated nuclei in which the chromatin granules were small. The fat was presumably derived from the nucleus since nuclei containing chromatin in a few large particles gave a good fat reaction. As degeneration proceeded albumin was let into the nucleus by a process of osmasis, and then the chromatin material formed small particles giving no fat reaction. Clumps of pigment material represented the remains of degenerated nuclei. The iron reaction in this tumor was positive.

GEORGE J. RUKSTINAT.

LIVER CELLS IN THE SPLEEN OF A PREMATURE INFANT. P. SCHNYDER, Centralbl. f. allg. Pathol. u. path. Anat. **37**:49, 1926.

In a 7 months infant girl, who lived five days, a group of liver cells 185 by 120 mikrons containing brown pigment, apparently bile, were found in the middle of the front part of the spleen.

GEORGE J. RUKSTINAT.

A CONTRIBUTION TO THE KNOWLEDGE OF NEURINOMATOSIS. G. L. DERMAN, Centralbl. f. allg. Pathol. u. path. Anat. **37**:52, 1926.

A walnut-sized dural tumor causing compression of the spinal cord removed at an operation contained endothelial elements in whorls, rosets and papillae, and large collections of these were separated by collagenic connective tissue.

Tumors were removed from the corium and subcutaneous tissue, cherry to plum size, white-gray, and somewhat transparent and shiny. Histologically elongated cells were found in palisade arrangement; myelin sheaths were not demonstrable but fat was found in places suggesting that seen in the cells of Schwann.

GEORGE J. RUKSTINAT.

THE PROCESS OF DEPOSITION OF COLLOIDAL MATERIALS IN THE ORGANISM. J. T. TEPLOW, Arch. d. sc. biol. **25**:292, 1925.

The author studied the deposits of colloidal carmine in rabbits after intravenous injection, and followed their disappearance for several months. Accumulations appeared first diffusely in the connective tissue, especially in the fibrous capsules of the organs, walls of the arteries, intestines and urinary passages. At the same time, the dead cells became colored. The carmine disappeared rapidly from the connective tissue, but remained long in the dead cells, especially in bone and teeth. Granules of the dye were also found in the cells of the reticulo-endothelial system, Kupffer cells, reticular cells of the bone marrow and spleen, and macrophages. It is excreted through the liver and kidneys.

Particles of China ink were deposited in the Kupffer cells and in the spleen a few minutes after injection, later in the bone marrow, and almost none in the other reticulo-endothelial cells. There were no diffuse accumulations in the connective tissue. The reaction to China ink is confined to the hepatic and splenic systems, while the entire reticulo-endothelial system is involved in the reaction to colloidal particles of carmine.

B. R. LOVETT.

THE INFLUENCE OF LOCAL LESIONS OF THE ARTERIAL WALLS ON THE FORMATION OF LIPOID DEPOSITS AT THE INJURED SITES. A. A. SALOVIEW, Arch. d. sc. biol. **25**:291, 1925.

Although the general etiology of atherosclerosis, the alteration of cholesterin metabolism, is considered to be definitely established, the local factors involved in accumulations of cholesterin in the vessel walls are not yet clear. The author produced lesions in the arteries of rabbits by burning, and at various times before and after the operation, introduced solutions of cholesterin into the stomach. The results were as follows: If the cholesterin was introduced some time after the operation when regeneration had occurred, no accumulations were found at the site of the lesions; but if it was given before or soon after the injury, cholesterin deposits were found in and around the lesion. It appears, therefore, that accumulation of lipoids at injured parts of the arterial walls depends on the state of the pathologic process at the time of the cholesterinemia.

B. R. LOVETT.

FAT EMBOLISM. R. WEINGARTEN, Schweiz. med. Wchnschr. **56**:248, 1926.

Weingarten examined 100 cadavers for fat embolism, and found a small amount in sixteen, a large amount in three and none in the remainder. In eleven of the positive cases, there was a probable or definite cause, such as injury or operation. One patient had had an injection of camphor in oil before death. Five infants who were given injections of oil before death, showed fat embolism at necropsy. The author found no relationship between nephritis and fat embolism, and thinks the condition is always due to some outside cause, possibly unnoticed traumas.

B. R. LOVETT.

MORPHOLOGIC APPEARANCES IN INFLAMMATORY PROCESSES IN LEUKEMIA. KURT BICKHARDT, Folia haematol. **32**:83, 1925.

Leukemic patients react to inflammatory stimuli in essentially the same manner as well persons, but in the exudate the pathologic cells of the blood take the place of the polymorphonuclear leukocytes.

Pathologic Chemistry

BIOCHEMICAL DIFFERENCES BETWEEN SEXES IN GREEN PLANTS. SOPHIA SATINA and B. F. BLAKESLEE, Proc. Nat. Acad. Sc. **12**:197, 1926.

Oxigenase, peroxidase, tyrosinase, acid and tannin production, and reduction of potassium permanganate, sodium selenate or methylene blue were greater in the female plants, and all female plants reacting thus characteristically to these tests had yellowish alcoholic extracts and typical Manoilov reactions. The age of the leaves was an important factor. The upper and lower leaves of a male *Cannabis*, for example, gave the male and female reactions, respectively. The midrib of a mature *Rhumex* leaf gave the male, and the blade the female reaction; and the sexual organs of the flowers gave corresponding tests. Of 172 tested cases, over 90 per cent correct identifications were obtained with the Manoilov test.

ETHEL B. PERRY.

THE MUCOR PARASITE PARASITELLA IN RELATION TO SEX. SOPHIA SATINA and A. F. BLAKESLEE, Proc. Nat. Acad. Sc. **12**:202, 1926.

In the study of *Mucors* that are parasitic and form galls with other *Mucors*, there was a tendency for one sex of the parasite to react preferentially with a single sex of the host. For example *Parasitella IV* of their series tended to form galls only with the (—) *Absidia*. Gall formation was not, however, considered to be a truly sex limited relation.

ETHEL B. PERRY.

THE DETERMINATION OF SUGAR IN BLOOD AND IN NORMAL URINE. O. FOLIN, J. Biol. Chem. **67**:357, 1926.

The alkaline copper tartrate reagent of the original Folin-Wu method is modified in such a way as to eliminate partly its reduction by nonglucose constituents of blood and urine. The acid molybdate reagent is improved so as to produce a deeper color with a given amount of cuprous oxide than formerly and to give a more dependable proportionality between readings of the standard at different color levels.

ARTHUR LOCKE.

THE RELATION OF THE CHOLESTERIN—CHOLESTERIN ESTER EQUILIBRIUM IN THE BLOOD AND SERUM TO LIVER FUNCTION. S. J. THANNHAUSER and H. SCHABER, Klin. Wchnschr. **5**:252, 1926.

The liver exerts a pronounced effect on the cholesterin—cholesterin ester equilibrium in the blood due to the presence in its cells of cholesterin esterases. Disorders of the liver parenchyma may possibly be recognized by an increase of the cholesterin—cholesterin ester ratio in the blood.

ARTHUR LOCKE.

THE ORIGIN OF OXALIC ACID (OXALURIA). F. PICCININNI and E. LOMBARDI, Klin Wchnschr. **5**:260, 1926.

An especial strain of *B. coli,* capable of forming oxalic acid, is found in the intestinal flora of persons with oxaluria. The strain may be isolated and produces oxalic acid in vitro when cultivated in potato broth. When administered by mouth, it produces oxaluria in persons free from this affection.

ARTHUR LOCKE.

VITAL STAINING OF PERFUSED LIVER. G. SEEMANN, Beitr. z. path. Anat. u. z. allg. Pathol. **74**:332, 1925.

The perfusion of the frog's liver with colloidal lithium carmine or trypan-blue in Locke-Ringer solution led to diffuse faint staining of the cellular elements but not to true vital storage of the dyes. The addition to the perfusing solution of colloids like gelatin, egg-albumin or blood serum did not alter the results. Perfusion with carmine in suspension in Locke-Ringer solution led to vital storage of the dye by the Kupffer cells.

O. T. SCHULTZ.

VITAL STAINING OF LUNG. G. SEEMANN, Beitr. z. path. Anat. u. z. allg. Pathol. **74**:345, 1925.

Oeller has recently claimed that the injection of chicken corpuscles into sensitized guinea-pigs is followed by rapid proliferation of capillary endothelium and of the perivascular cells of the lung. Aschoff has ascribed to the lung the important function of removing from the circulation and of digesting histiocytes and reticulo-endothelial cells which enter the circulation from the liver, but he does not believe that the capillary endothelium takes any part in the phagocytic process. Seeman investigated these and related questions by means of injection of trypan blue and saccharated iron oxide into mice. Histiocytes and the alveolar epithelium of the lung are phagocytic to trypan blue. The coarser iron oxide is not removed from the blood by phagocytic cells of the lung but is held only mechanically. Intratracheal injection of iron oxide leads to immediate storage of the material within alveolar epithelia; not all of the latter, however, take part in this reaction. Seemann calls attention to the normal presence of sheaths of large and small lymphoid elements about the vessels of the lung in both the mouse and the guinea-pig. The subpleural zone of the lung normally contains alveolar epithelium which has become swollen by the presence of fat and dust particles. The perivascular proliferative reaction described by Oeller could not be induced by intravenous injection of hemoglobin, and Seemann intimates that Oeller was dealing with normal variations within the lung.

O. T. SCHULTZ.

Microbiology and Parasitology

STUDIES ON VITAL STAINING IN EXPERIMENTAL TUBERCULOSIS. R. H. JAFFÉ, Am. Rev. Tuberc. **13**:97, 1926.

Rabbits were most intensively stained vitally by injections with India ink and with saccharated iron oxide. The iron was found in the Kupffer cells, in cells in the splenic sinuses, in the reticular cells of the splenic pulp and the malpighian bodies, in the endothelial and reticular cells of the bone marrow, in

some cells in the alveolar septums of the lung and in the endothelial cells of the sinus in lymph nodes. Iron was never found in capillary endothelial cells, nor in cells lying in the lumen of blood vessels. Ink is deposited in small thrombi in small pulmonary vessels. The endothelial cells of alveolar capillaries never contain ink. Ink is stored in the Kupffer cells and in the periportal connective tissues, in cells of the splenic pulp and in the malpighian bodies, in the endothelial cells of the spleen sinuses, in reticular and endothelial cells of the bone marrow, in the kidney tufts and surrounding capillaries. After the animals were so intensively stained, they were infected with bovine bacilli. Tubercle formation took place in the usual manner. The intensity of staining in the tubercles is in direct proportion to the number of preexistent stained cells at the site of tubercle formation. The decrease of staining in growing tubercles is not a result of a diminished vitality of the epithelioid cell, but is due to the fact that the proliferating cells in the avascular tubercles are removed from the source of the dye.

MAX PINNER.

THE REACTION AND GROWTH CURVES OF CULTURES OF TUBERCLE BACILLI. R. R. HENLEY, Am. Rev. Tuberc. **13**:107, 1926.

Comparing growth and reaction curves of human and bovine tubercle bacilli, it was found that the more abruptly and higher the growth curves rose during the early weeks of incubation, the earlier and more sharply the reaction curves fall after reaching the maximum. The final reaction of cultures with heavy growth approached p_H 5, but never fell below that point. The acid produced after eight weeks was dependent on the amount of bacillary growth after three weeks.

MAX PINNER.

UNSUCCESSFUL ATTEMPTS TO CURE OR PREVENT TUBERCULOSIS IN GUINEA-PIGS WITH DREYER'S DEFEATED ANTIGEN. M. NEVIN, F. R. BITTMAN and E. L. HAZEN, Am. Rev. Tuberc. **13**:114, 1926.

Treatment with Dreyer's diaplyte vaccine produced no beneficial effect in tuberculous guinea-pigs. It was generally followed by suppurative lesions, which healed only in a few instances. The treated animals died earlier than the nontreated controls.

MAX PINNER.

A PRELIMINARY REPORT OF EXPERIMENTAL IMMUNIZATION AGAINST TUBERCULOSIS IN THE GUINEA-PIG BY THE USE OF "OLEO-VACCINE." W. J. TULLOCH, Tubercle **7**:218, 1926.

Exposure of dry living tubercle bacilli to olive oil having an acid value of 0.75 per cent—reckoned as oleic acid—for four days at 37 C. caused the death of the bacilli. At least as much bacillary substance as 1.5 mg. (wet weight) per cubic centimeter of oil could be thus treated with the assurance that the product so obtained was noninfective for guinea-pigs. Guinea-pigs treated with this oleovaccine did not develop tuberculosis after the infection with oil treated tubercle bacilli of very low virulence which infected only two of three normal controls.

MAX PINNER.

FURTHER OBSERVATIONS UPON TUBERCULOSIS INOCULATA OF THE GUINEA-PIG. G. R. Ross and W. J. TULLOCH, Tubercle **7**:265 and 321, 1926.

The bactericidal effect of various oils on tubercle bacilli is not dependent on their iodine value, nor on their being glycerides. Vaccines of tubercle bacilli killed in olive oil are even in large doses innocuous to guinea-pigs; whether they have any protective or curative effect is still a matter of experimental investigation.

MAX PINNER.

ROCKY MOUNTAIN SPOTTED FEVER. R. R. PARKER and R. R. SPENCER, Pub. Health Rep. **41**:461, 1926.

The data as presented show the following: 1. Although of known infected adult ticks the majority of those containing rickettsia were infective, of each lot tested a small group of noninfective ticks contained rickettsia morphologically identical, while still another small group was infectious although the tick smears were entirely free from organisms. 2. Of wild ticks from a known infected area, a considerable proportion contained rickettsia indistinguishable from those associated with spotted fever, and that the smear and inoculation results of such ticks were parallel with those of the known infected group. 3. A small proportion of wild ticks from a supposedly uninfected area contained similar rickettsia, but none caused infection.

It is difficult to account for the noninfective rickettsia which were present in part of the known infected, laboratory reared ticks and which exhibited a morphology identical with that of the rickettsia in fever-producing ticks of the same group. They may represent an avirulent phase of the spotted fever virus, although the nonpathogenic nature of these bodies cannot, of course, be ruled out. This accords with previous observations of tick virus in a similar lot of known infected ticks by which was demonstrated various degrees of virulence for guinea-pigs ranging from a noninfective or an immunizing phase in unfed, aestivating or hibernating ticks to an active highly virulent phase following feeding. The term "reactivation" has been used to designate this transition, which has repeatedly been observed in known infected lots. For example, in recently infected larvae, the virus is present but is noninfective unless massive doses are used (five engorged larvae rarely infect; twenty-five usually, but not always cause infection, often of a mild character); in the resultant unfed hibernating nymphs the virus is present in either a noninfective or an immunizing phase, but in the fed nymphs it has acquired marked virulence; a noninfectious or immunizing phase is again encountered in the resultant unfed, aestivating or hibernating adults, but in the fed adults a high degree of virulence has been reacquired.

In presenting these observations it is realized that the relatively small part of the tissue of a tick represented by smear preparations cannot be taken as absolute evidence of the absence of rickettsia from the entire tick. However, it is at least reasonable to believe that they were few, since the test ticks had all ingested blood, and the rickettsia had thus been afforded, as we have shown, the most favorable conditions for multiplication and distribution throughout the various tissues. There is, of course, the possibility that they were present in an unrecognized form.

AUTHORS' SUMMARY.

FATE OF BACT. TYPHOSUM AND OTHER ORGANISMS IN SEGREGATED VEIN AND IN GENERAL CIRCULATION OF NORMAL AND IMMUNIZED RABBITS. R. G. MILLS and G. M. DACK, J. Infect. Dis. **38**:222 (March) 1926.

A technic has been developed for studying separately the quantitative relations of the humoral and cellular destruction of bacteria in vivo. Marked reduction in bacterial counts was noted in samples taken from the heart blood, segregated vein, heparinized and citrated blood, serum and Ringer's solution. The rate of destruction in all except the general circulation followed fairly closely the monomolecular type of reaction curve. The rate in the general circulation was more rapid, conforming to the bimolecular equation. In the general circulation there is the added factor, absent in the other mediums, of phagocytosis, and perhaps agglutination and filtration.

With typhoid bacilli the rate of destruction was approximately the same in the in vitro experiments as in the segregated vein, while with staphylococci there was a marked difference between the two. This suggests that the relative importance of humoral and cellular destruction of bacteria is different for different species of organisms.

B. R. LOVETT.

EXPERIMENTAL METHOD FOR STUDY OF BACTERIAL FLORA OF GASTRO-INTESTINAL TRACT. LLOYD ARNOLD, J. Infect. Dis. **38**:246, 1926.

By fixing certain segments of the small intestine to the anterior abdominal wall, specimens can be obtained for both physiologic and pathologic study

AUTHOR'S SUMMARY.

BACTERIAL FLORA AND HYDROGEN ION CONCENTRATION OF DUODENUM. LLOYD ARNOLD and LOUIS BRODY, J. Infect. Dis. **38**:249, 1926.

When the normal reaction of the contents of the duodenum and upper jejunum is changed from a slightly acid (p_H 5 to 6) to a neutral or alkaline reaction (p_H 7 to 8), there is a change in the bacterial flora of this region.

When the contents of the duodenum and upper jejunum are neutral or alkaline in reaction, the bacterial flora resemble that found in the ileum and cecum.

The normal duodenal flora is not changed by tying off the pancreatic ducts.

The maintenance of the normal hydrogen ion concentration of the contents of the duodenum and upper jejunum is dependent to a great extent on normal gastric secretory function.

AUTHORS' SUMMARY.

EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT: VII. CARRIERS OF BACTERIUM LEPISEPTICUM. LESLIE T. WEBSTER, J. Exper. Med. **43**:573, 1926.

Rabbits with no previous exposure to *Bact. lepisepticum* were given, intranasally, a known dose of one strain or another of this organism or of *B. bronchisepticus*. The results give grounds for the following conclusions.

Results referable to the fate of the bacteria: Highly virulent strains of *Bact. lepisepticum* are incapable of vegetating indefinitely in the nasal passages of surviving rabbits. But from the organism of high virulence, variants of low pathogenicity may develop and persist for a few days. Less virulent, mucoid strains of *Bact. lepisepticum* and strains of *B. bronchisepticus* are capable of surviving indefinitely in the rabbit's nasal passages.

Results referable to the host: Rabbits refractory to infection under the conditions of the experiment usually show no lesions of the nasal passages other than those to be encountered in control rabbits. These may show slight microscopic, focal, chronic, inflammatory lesions of the nasal passages.

The animals which become chronic "healthy" carriers, as a result of the infection, and those which show clinical symptoms of snuffles all develop well marked, chronic inflammatory lesions of the nasal passages.

Those markedly susceptible animals which succumb to pneumonia caused by the injected organism all show an acute type of inflammatory lesion of the nasal passages.

AUTHOR'S SUMMARY.

EXPERIMENTAL STUDIES ON THE FILTRABLE TUBERCULOSIS VIRUS AND ITS PASSAGE THROUGH THE PLACENTA. F. ARLOING and A. DUFOURT, Bull. Acad. de méd., Paris **95**:163, 1926.

Tuberculous material from children, such as tuberculous skin lesions, tuberculous lymph glands, spinal fluid from tuberculous meningitis, pleural pus and ground tuberculous organs, were passed through Chamberland filters L3 under from 30 to 50 pounds of pressure. These filtrates never yielded any cultures of tubercle bacilli and were injected into guinea-pigs. Sixteen of thirty filtrates produced different types of lesions. In fourteen cases, "une tuberculisation d'allure très spéciale" was obtained, no ulcer or regional lymph gland swelling corresponding to the site of infection. After some months the animals died; they were very emaciated but showed neither caseating nor granular lesions; the lymph glands were either normal or slightly enlarged; they showed no specific tissue changes, but contained numerous acid-fast rods. In two other animals nodular and caseous lesions were found in the lymph glands and internal organs; but no primary lesion. In a guinea-pig embryo at the end of the second third of pregnancy, whose mother was injected with a filtrate as mentioned above, numerous acid-fast rods were found in a lumbar lymph gland. In the human fetus or in the new-born from tuberculous mothers, in their placentas or in their umbilical blood, a filtrable virus has never been found. Further studies on the acid-fast rods recovered from animals which had been injected with filtrates are not reported.

MAX PINNER.

ENDOBRONCHIAL INOCULATIONS AND EXPERIMENTAL PULMONARY TUBERCULOSIS. G. PETIT, L. PANISSET and P. KFOURY, Bull. Acad. de méd., Paris **95**:169, 1926.

Following endobronchial inoculations of tubercle bacilli by means of a soft catheter, dogs and rabbits develop localized caseous pneumonia of the acute type, which soon leads to cavity formation. These cavities resemble the type usually found in man; they are located close to the pleura and sometimes give rise to spontaneous pneumothorax.

MAX PINNER.

TREATMENT OF SUPPURATING INFLAMMATIONS WITH FILTRATES ACCORDING TO THE BESREDKA METHOD. N. N. BOURDENKO and N. L. GIVAGO, Ann. de l'Inst. Pasteur **40**:232, 1926.

In thirty-eight cases of chronic and acute suppurating infections apparently favorable results were obtained by the direct application to the infected area of sterile filtrates of broth cultures of the organisms isolated from it. Staphylo-

coccus and streptococcus were the predominating organisms, and some use was made of filtrates of stock cultures but with less favorable results. It is stated that in general there was acceleration of healing and rapid cicatrization.

G. B. RHODES.

COAGULATION OF EGG YOLK BY A MICROBE DIASTOSE. E. LAGRANGE, Ann. de l'Inst. Pasteur **40**:242, 1926.

Vitellase is the name given to a diastase which coagulates but does not digest the vitelline of egg yolk. The calcium ion does not enter into the process as it does in the coagulation of milk or plasma. *B. sinicus,* some related organisms and molds in general have this diastase which is of industrial importance.

G. B. RHODES.

NEW INVESTIGATION OF BACTERIAL FLAGELLA. K. YOKOTA, Centralbl. f. Bakteriol. **95**:261, 1925.

Typhoid bacilli grown in water of condensation agglutinate in larger clumps and more rapidly than bacilli grown on agar slants. This corresponds with difference in growth of flagella. Shaking a suspension of bacilli until the flagella are separated diminishes the amount and speed of agglutination. Complete and rapid agglutination is attained only by the presence of bacilli and flagella. If a suspension of flagella, separated from the bacteria, is formed and mixed with immune serum, agglutination of the flagella takes place, showing that the flagella by themselves have the power of being agglutinated. Heating to 65 degrees for ten minutes causes the clumps to disappear, by destroying the bodies of the flagella. This is the probable explanation of the decreased agglutinability of typhoid bacilli after heating. These facts should be considered in studying the agglutination reactions of bacteria provided with flagella.

B. R. LOVETT.

DROPLET VS. DUST INFECTION IN TUBERCULOSIS. W. STRAUSS, Ztschr. f. Hyg. u. Infectionskrankh. **105**:416, 1925.

It seemed for the last years that Flügge's theory about the predominance of droplet infection in tuberculosis had been quite generally accepted. B. Lange and his co-workers claimed recently, on the basis of extensive experimental work, that fine dust particles are much more dangerous carriers of the infection than droplets, and that droplets may carry tubercle bacilli in the lungs only when they are not bigger than 20 microns, or exceptionally 100 microns. Most of the sputum droplets are supposed to be from 100 to 500 microns in diameter. He believes, therefore, that infection through sputum droplets is a "rare happening." Strauss takes exceptions to these statements; he disputes the conclusiveness of Lange's observation on bent glass tubes representing a model of the bronchial tree, and shows the fallacies in measuring the diameter of droplets after they have settled on a glass slide. He presents apparently valid evidence for his claim that the suspended droplets are not more than half the size of the measured droplets on glass slides. According to his corrected measurements, the majority of cough droplets have a diameter of from 70 to 85 microns, a size which may easily enter the lower parts of the respiratory tract. This, taken with the plentiful evidence that guinea-pigs

can be infected by exposure to a coughing tuberculous patient, leads the author to emphasize again the prime importance of droplets as the infecting agent. He points out that guinea-pigs which breathe through the nose exclusively are not as easily infected as man.

MAX PINNER.

OBSERVATIONS ON THE POSSIBILITY TO CHANGE ACID-FAST SAPROPHYTES INTO TRUE TUBERCLE BACILLI. S. ZLATOGOROFF, M. ZECHNOWITZER and M. KOSCHKIN, Ztschr. f. Hyg. u. Infectionskrank. **105**:583, 1925.

These experiments were made on Moeller's Timothee bacillus, Rabinowitsch's bacillus and on the smegma bacillus. Attempts were made either to influence the bacilli so as to increase their virulence, by growing them on lactic acid containing mediums (Much) and by growing them on vitamin-free mediums (Setti); or by weakening the resistance of the infected animals by keeping them either on a deficient diet or on a vitamin-poor or vitamin-free diet. The animals were infected serially by subcutaneous or intraperitoneal injections. As a whole, the experiments were unsuccsesful. Only the tubercle bacilli grown on lactic acid mediums acquired the tendency to spread throughout the animal's body, without producing definite pathologic lesions or the symptoms of toxemia.

MAX PINNER.

MIXED INFECTION IN CHRONIC PULMONARY TUBERCULOSIS. J. ABAKELIA, Ztschr. f. Tuberk. **44**:306, 1926.

Blood and sputum of seventy-one patients were examined. In forty-one patients the blood contained micro-organisms, such as streptococci and staphylococci, both of the hemolytic and nonhemolytic type. In most cases the same bacteria were found in the sputum. Hemolytic germs were found frequently even in the blood of afebrile patients, and in those with benign productive processes.

MAX PINNER.

THE HEMOCLINIC STATUS OF PATIENTS WITH SURGICAL TUBERCULOSIS. A. SCHANIN and W. CHRENNIKOW, Beitr. z. klin. Tuberk. **62**:759, 1926.

The study on sixty tuberculous patients and ten normal persons yielded the following results: The electric conductivity and the degree of dissociation, the albumin-globulin ratio, the sedimentation time of the red cells, the surface tenson and the calcium content of the serum decrease with the severity of the process.

MAX PINNER.

THE EXCRETION OF TUBERCLE BACILLI IN HUMAN BILE. H. ROSENHAGEN, Beitr. z. klin. Tuberk. **62**:764, 1926.

In the bile of eleven patients, collected through the duodenal tube, tubercle bacilli were demonstrated three times. Only one of these three patients had tubercle bacilli in the sputum.

MAX PINNER.

FUNGOUS CASTS IN THE URINE. E. GUNDERSEN, Norsk Mag. f. Lægevidensk. **87**: 124, 1926.

No albumin, pus or blood elements were found to explain the dysuria in a man. but casts were found that evidently were formed by a penicillium fungus.

DIPHTHERIA ACCOMPANYING ACTIVE IMMUNITY. M. HAIDVOGL, München. med. Wchnschr. **73**:358, 1926.

Twelve of thirty children ill with diphtheria gave a negative Schick reaction. Five had a blood antitoxin content of 0.04 to 0.45 antitoxin units.

ARTHUR LOCKE.

Immunology

THE STANDARDIZATION OF TUBERCULIN. J. D. ARONSON, Am. Rev. Tuberc. **13**:263, 1926.

Ten samples of old tuberculin, greatly varying in strength, were titrated by intracutaneous reactions in tuberculous guinea-pigs (Lewis and Aronson), by their toxicity for tuberculous guinea-pigs (Koch's method), by complement fixation (Watson and Heath) and by precipitin tests (Dreyer and Vollum). The first two methods gave comparable results, whereas the two serologic methods differed widely in their results from those obtained by biologic methods.

MAX PINNER.

RESISTANCE TO TUBERCULOUS INFECTION OF GUINEA-PIGS RENDERED SKIN SENSITIVE WITH DEAD TUBERCLE BACILLI. L. DIENES and E. W. SCHOENHEIT, Am. Rev. Tuberc. **13**:379, 1926.

Guinea-pigs that were sensitized to tuberculin by injections of an emulsion of ether and alcohol extracted tubercle bacilli were not more resistant to a subsequent tuberculosis infection than unprepared control animals.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: 1. A NON-PROTEIN MEDIUM SUITABLE FOR THE PRODUCTION OF TUBERCULIN IN LARGE QUANTITY. E. R. LONG and F. B. SEIBERT, Am. Rev. Tuberc. **13**:393, 1926.

The following medium of known chemical compositoin is recommended for the growth of tubercle bacilli: asparagine, 5 Gm.; ammonium citrate, 5 Gm.; potassium acid phosphate, 3 Gm.; sodium carbonate (anhydrous), 3 Gm.; sodium chloride, 2 Gm.; magnesium sulphate, 1 Gm.; ferric ammonium citrate, 0.05 Gm.; glycerol, 50 Gm.; water, 1,000 Gm. This represents a well buffered medium which does not require titration. About 100 to 150 Gm. of moist tubercle bacilli (H37 or R1) will grow on 1 liter in from four to six weeks. During the growth of tubercle bacilli on this medium, there appear simultaneously protein substances and tuberculin.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: II. PRECIPITATION WITH ACETIC ACID AND OTHER ACIDS. E. R. LONG and F. B. SEIBERT, Am. Rev. Tuberc. **13**:398, 1926.

In precipitating the culture broth (described in the preceding article) with acetic or phosphoric acid, the maximum of precipitate was obtained at a p_H of 4, which is the iso-electric point of the precipitate, which has a high

tuberculin protency. In this procedure not all of the protein present is precipitated; the filtrate still contains protein and active tuberculin. The tuberculin activity may accordingly be associated with more than one protein compound.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: III. EXPERIMENTS ON DIALYSIS. F. B. SEIBERT and E. R. LONG, Am. Rev. Tuberc. **13**:404, 1926.

If culture broth containing potent tuberculin is subjected to dialysis, only slight traces, if any, of tuberculin pass the membrane. The same is true of the proteins. A carbohydrate, however, dialyzes with the inorganic and odoriferous substances, indicating that these substances are not associated with the active principle in tuberculin.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: IV. AMMONIUM-SULPHATE PRECIPITATION OF THE PROTEINS OF TUBERCULIN. F. B. SEIBERT and E. R. LONG, Am. Rev. Tuberc. **13**:408, 1926.

Of the various protein precipitants used in these studies, ammonium sulphate in from three-quarters to full saturation is the only one which precipitates the active principle of tuberculin completely. The ammonium sulphate precipitate could be divided into two fractions; one soluble in water, the other insoluble in water but soluble in weak sodium hydroxide. The former contains a potent tuberculin; the latter elicits only slight reactions, and these may be due to contaminations with the water-soluble fraction. The water-soluble fraction can be separated into two fractions, one being coagulable by heat, the other not; both containing active tuberculin. While no active substances dialyze from untreated culture broth, it was regularly found that a considerable amount of tuberculin does dialyze from the water-soluble ammonium sulphate precipitate, suggesting the possibility that the first cleavage products of proteins (proteoses) may be active.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: V. THE EFFECT OF PROTEOLYTIC ENZYMES ON TUBERCULIN PROTEINS AND THE ACTIVITY OF TUBERCULIN, F. B. SEIBERT, Am. Rev. Tuberc. **13**:431, 1926.

The active principle in the water-soluble, noncoagulable fraction is destroyed by the enzymatic action of trypsin in alkaline solution; it is not destroyed in neutral solution, because trypsin in neutral solution attacks primarily the proteoses. Erepsin does not destroy the active principle. An analysis of the behavior of the active tuberculin principle when exposed to various enzymes suggests strongly the conclusion that it is definitely associated with the whole protein molecule.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: VI. ACID HYDROLYSIS OF TUBERCULIN. E. R. LONG, Am. Rev. Tuberc. **13**:441. 1926.

A highly potent tuberculin was submitted to acid hydrolysis at various degrees of acidity. Only those samples which after hydrolysis still contained material precipitable by trichloracetic acid gave a positive skin test in an

allergic subject. The samples giving no precipitate with trichloracetic acid still contained substances (proteoses) precipitable by ammonium sulphate.

MAX PINNER.

THE CHEMICAL COMPOSITION OF THE ACTIVE PRINCIPLE OF TUBERCULIN: VII. THE EVIDENCE THAT THE ACTIVE PRINCIPLE IS A PROTEIN. E. R. LONG and F. B. SEIBERT, Am. Rev. Tuberc. **13**:448, 1926.

In this last article in the series the authors summarize the evidence for their opinion that the active principle in tuberculin is a whole protein as follows: "The active principle of untreated tuberculin is a colloidal substance which does not pass through animal and vegetable membranes, except in traces. A colloid giving all the usual protein tests may be precipitated by acids at its iso-electric point, p_H 4. This substance is highly active in the tuberculin test. Precipitation of protein is not complete by this method, however, and the filtrate, containing some protein, also retains tuberculin activity. Saturation of tuberculin solutions with ammonium-sulphate precipitates all detectable protein. The precipitate is the most highly active of all tuberculin preparations we have obtained. The filtrate contains no protein, and shows no tuberculin activity. The most specific reagents for protein at our disposal, proteolytic enzymes, lessen or destroy tuberculin activity at the same time that they disrupt protein present. As whole protein decreases and protein cleavage products accumulate in solutions of tuberculin subjected to proteolytic enzyme action, tuberculin activity decreases or disappears. When whole protein disappears on acid hydrolysis activity is lost. As long, however, as whole protein is present, activity remains. There is evidence that a protein product of smaller molecular weight than "whole protein" may be active. Active principle in traces dialyzes. "Proteose" obtained by ammonium-sulphate precipitation, after "whole protein" has been precipitated by trichloracetic acid, has some activity. These results signify that activity is associated primarily with the whole-protein molecule, but persists in spite of a certain amount of disintegration of the molecule.

MAX PINNER.

STUDIES IN TUBERCULOSIS: VII. ACTIVE PRINCIPLES OF TUBERCULIN PREPARED FROM NON-PROTEIN SUBSTRATES. F. EBERSON, Am. Rev. Tuberc. **13**:454, 1926.

Three fractions have been prepared from a synthetic culture broth of tubercle bacilli: an alcohol-insoluble, an ether-insoluble and an ether-soluble fraction; the latter is claimed to be protein-free. As early as three days after infection these fractions are said to elicit specific skin reactions in guinea-pigs. They are capable of sensitizing normal guinea-pigs to the homologous and heterologous fractions. In juvenile patients they give diagnostic results far superior to old tuberculin. No opinion is offered as to the chemical nature of the active and sensitizing principle.

MAX PINNER.

ATTEMPTS AT THE ACTIVE IMMUNIZATION OF GUINEA-PIGS BY TUBERCULOUS URINARY ANTIGENS. J. J. ENRIGHT, Am. Rev. Tuberc. **13**:463, 1926.

An attempt was made to immunize guinea-pigs actively with the tubercle bacillus antigens which are supposed to be excreted in the urine of tuberculous patients. Although there appears to be some slight evidence that a certain

degree of sensitization was obtained, the author is well aware of the fact that this question cannot be definitely answered unless a much larger number of animals are subjected to the same procedure.

MAX PINNER.

ALLERGIC IRRITABILITY: III. THE INFLUENCE OF CHRONIC INFECTIONS AND OF TRYPAN BLUE ON THE FORMATION OF SPECIFIC ANTIBODIES. PAUL A. LEWIS and DOROTHY LOOMIS, J. Exper. Med. **43**:263, 1926.

The allergic irritability of the guinea-pig (capacity of the animal to react to antigenic substances) is increased by infection with *Bacillus abortus* and a streptococcus, by the dead tubercle bacillus, and by intensive treatment with trypan blue, respectively. The effect of these influences, while definite, is less pronounced than that previously found for infection with the tubercle bacillus. The production of anti-sheep hemolytic amboceptor was used as the test reaction in these cases.

The allergic irritability of the guinea-pig with reference to antityphoid agglutinin is increased by infection with the tubercle bacillus.

The allergic irritability of the rabbit with reference to anti-sheep hemolytic amboceptor is increased by an infection of suitable severity with the tubercle bacillus.

In the guinea-pig the curve of antibody production is complex. Its peculiarities are developed during the production of antityphoid agglutinins as well as that of anti-sheep hemolytic amboceptor. In the latter case injections of antigen subsequent to the first give rise to a curve of production unchanged in form but somewhat affected in the time relations.

The effects of infection with *Bacillus tuberculosis* on allergic irritability with reference to anti-sheep hemolytic amboceptor are operative throughout a course of immunizing treatments. The successive increases due to the cumulative effect of repeated doses of the antigen are developed on a higher level. The end-result is that the animal with increased irritability furnishes more antibody not only in response to the initial injection of antigen as previously described, but an absolute increase over the amount attainable by a comparable number of treatments in series. That portion of the final result contributed by the increase in allergic irritability appears to be no less, and may even in instances be somewhat more than that due to the earlier doses of the specific antigen.

AUTHORS' SUMMARY.

ANAPHYLACTIC SHOCK CAUSED BY ANTIBODY IN ANIMALS SENSITIZED BY ANTIGEN REVERSED PASSIVE ANAPHYLAXIS. EUGENE L. OPIE and J. FURTH, J. Exper. Med. **43**:469, 1926.

Anaphylactic shock occurs (in rabbits) when the usual procedure for the production of passive anaphylaxis is reversed; that is, when an animal previously treated with antigen receives the corresponding antiserum by way of the circulating blood.

This susceptibility to the action of anti-horse-serum produced by injection of antigen reaches maximum intensity after an interval of four hours, presumably required to permit penetration of the antigen in sufficient concentration into the tissues.

Desensitization to the action of a shock-producing dose of anti-horse-serum can be brought about by repeated small doses of the same antiserum.

Anaphylactic shock and local anaphylaxis manifested by the acute inflammation of an immunized animal when injected with the antigen used for

immunization (Arthus phenomenon) occur under analogous conditions; that is, when antigen and antibody meet within the tissues. The peculiar characters of these reactions are dependent on the site of entry of the irritating agent, which is the vascular system in one instance and tissue spaces in the other, and on the concentration of antigen and antibody within susceptible tissues.

Meeting of antigen and antibody within susceptible tissues is sufficient to explain the phenomena of local and general anaphylaxis so that it is unnecessary to assume the sudden formation of a toxic substance (anaphylatoxin).

AUTHORS' SUMMARY.

SPECIFIC RESISTANCE AS INFLUENCED BY NONSPECIFIC AGENTS. W. J. SCHATZ, J. Immunol. **11**:28 (Jan.) 1926.

The results obtained by the author support the idea that non-specific agents induce an effect which is equivalent to reinoculation by the excitant of the state in hand bringing it into play in a degree in ratio with the size of the dose employed, whereby specific resistance may be either elevated or depressed according as this effect is induced in a degree constituting beneficial or excessive stimulation.

S. A. LEVINSON.

QUALITATIVE RECEPTOR ANALYSIS: II. BACTERICIDAL SERUM ACTION AND QUALITATIVE RECEPTOR ANALYSIS. A. FELIX and L. OLITZKI, J. Immunol. **11**:31, 1926.

Immune serums which contain only small-flaking agglutinins (O-immune serums) possess a strong bactericidal power.

Immune serums which contain small-flaking and large-flaking agglutinins do not develop a stronger bactericidal action than O-immune serums with an equal content of small-flaking agglutinins; it is quite indifferent to the degree of bactericidal action whether large-flaking agglutinins, even to the greatest amount, are present or not.

Immune serums which contain large-flaking and small-flaking agglutinins lose entirely their bactericidal power by removing the small-flaking agglutinins (by absorption with 100 degree bacilli), in spite of the fact that the content of large-flaking agglutinins remains completely unchanged.

Immune serum of *B. enteritidis* Gaertner and immune serum of *B. typhosus* possess the same bactericidal amboceptor, and in both the small-flaking agglutinins are identical (Weil and Felix). The observation of Herzog and Schiff regarding cross-immunity between *B. typhosus* and *B. enteritidis* Gaertner finds in this way its experimental foundation.

Between *B. typhosus* and *B. paratyphosus* A and B there exist bactericidal group-amboceptors in the same way as there exist small-flaking group agglutinins.

Thus, it has been proved that in the typhoid-paratyphoid group the bactericidal amboceptors as well as the complement-binding immune bodies (Weil and Felix) are identical with the small-flaking (stabilotropic) agglutinins. They all owe their existence to the stable antigen of the bacteria (O-receptors).

The differences in the height of the titer in these three phenomena are to be found in the differences of the conditions of the experiment and of the sensitiveness in every one of these reactions.

The conclusions with reference to seroprognosis, serum therapy and preventive vaccination, which Felix drew from his investigation on typhoid patients, obtain their experimental proof from the present investigations.

AUTHORS' SUMMARY.

STABILITY OF STANDARD KAHN ANTIGEN. PEARL L. KENDRICK and DORA JENKS, J. Lab. & Clin. Med. **11**:369, 1926.

Kahn standard antigen was found to be practically unaffected by storage at 0, 21, 37 and 54 C. up to a period of nineteen weeks. It withstood the temperature of boiling water for at least an hour. After storage at 21 C. for one year, three different antigens gave results comparable to those given by freshly prepared antigen. An antigen stored for a year after cholesterinization gave practically the same results as the alcoholic extract stored for the same length of time but cholesterinized just before its use.

AUTHORS' SUMMARY.

THE PRINCIPLES OF IMMUNITY APPLIED TO INOCULATION AGAINST DIPHTHERIA. A. T. GLENNY, J. Hyg. **24**:301, 1925.

The injection of an antigen into a nonimmune animal acts as a primary stimulus. Injection into an immune animal acts as a secondary stimulus to which the response is greater and more rapid. The potential immunity produced by a single injection of toxin continues throughout life although the antitoxin content of the plasma is gradually lost. The natural antitoxin content of the plasma of normal persons is the result of recurrent stimuli by small amounts of toxin, and is variable. Guinea-pigs react consistently to the Schick test because they are used at such an early age that they have had little opportunity to acquire any degree of natural immunity. Any antigenic response depends on the immunity state of the individual as well as on the type of antigen. The true test of a toxoid or toxin-antitoxin preparation is the rate at which it immunizes the Schick reactors in a population with a high positive rate.

ARTHUR LOCKE.

PROPHYLACTIC IMMUNIZATION OF INFANTS AGAINST TUBERCULOSIS BY THE B C G VACCINE. A. CALMETTE, C. GUERIN, L. NEGRE and A. BOGUET. Rev. de la tuberc. **7**:5, 1926.

An extensive and detailed report is given on the experimental foundation and on the practical application of the B C G vaccine. The bacillus was originally a typical, pathogenic bovine tubercle bacillus, "some characteristics of which have been slowly and hereditarily modified through a long series of cultures in a very alkaline medium, rich in lipoids (oxlisle)." It has lost its tuberculogenic but has retained its antigenic properties. Animal experiments on small laboratory animals, on cattle and on anthropoid apes have demonstrated the innocuity and efficacy of this method. Immunized animals sometimes have a positive tuberculin reaction and sometimes not; this is said to be dependent not on the degree of immunity but on the presence of giant cells. In infants, too, the tuberculin reactivity does not follow any rule after this immunization.

In France 5,183 infants had been immunized by Jan. 1, 1926. No ill effect has been reported. Of this number, 586 infants were in familial contact with patients suffering from open cases of tuberculosis for a period of from six to eighteen months; the tuberculous mortality in this group was 1.8 per cent as compared to a mortality of at least 25 per cent in nonimmunized infants in contact with tuberculous patients.

The vaccine is said to be ineffective in already infected children or adults. The authors recommend, therefore, the immunization of each new-born child, particularly those in contact with tuberculous patients.

MAX PINNER.

IMMUNITY TO SPERM. L. BODNÁR and H. KAMNIKER, Deutsche med. Wchnschr. **51**:2119, 1926.

Bodnár and Kamniker mixed human sperma with the serum of more than 100 women. There were no definite results. The spermatozoa survived from two to thirteen hours. In the serum from three virgins the spermatozoa survived longer than in any one of the controls. They do not draw any definite conclusions from this small number.

CANCER IN THE HORSE. A. H. ROFFO, Bol. d. Inst. de med. exper. **1**:694, 1925.

Malignant tumors in horses are not frequent in the Argentine. Two are described, one in the nasal cavity and one on the vulva — both of the pavement epithelium type.

B. R. LOVETT.

GROUP SPECIFIC ISO-OPSONINS IN HUMAN SERUM. F. SCHIFF, Med. Klin. **21**: 1238, 1925.

That human serum may contain substances that promote phagocytosis of human red corpuscles (isohemopsonins) has been known since about 1906, but the exact relation of these substances to iso-agglutinins and isolysins has not been determined. Schiff shows that the blood from different persons may be divided into four groups by the study of the effect of iso-opsonins. The results are analogous to those of iso-agglutination, but iso-opsonins are not present regularly in as easily demonstrable quantities as the iso-agglutinins. Schiff found that serum rich in isolysin is certain to contain iso-opsonin. Iso-opsonin does not stand in any relation to disease; it is present in the serum of healthy persons, but this does not mean that abnormal iso-opsonin perhaps may not appear under pathologic conditions.

THE VALUE OF COLIAGGLUTINATION FOR THE CLINICAL DIAGNOSIS OF COLI INFECTIONS. V. HOFFMAN and K. L. PESCH, Klin. Wchnschr. **4**:2345, 1925.

Coliagglutination has not a practical significance for the diagnosis of coli infections. The occurrence of agglutination depends more on the agglutinability of the coli strain used than on the presence of coliagglutinins in the patient's serum.

ARTHUR LOCKE.

ISOHEMAGGLUTINATION. G. H. SCHNEIDER, Klin. Wchnschr. **4**:2383, 1925.

No toxemia is found to accompany pregnancy when the blood group of mother and child coincide. A divergence in blood group is a disposition toward eclampsia.

ARTHUR LOCKE.

THE BIOLOGIC SIGNIFICANCE OF THE LIPOIDS. HANS MUCH, München. med. Wchnschr. **72**:2089 and 2143, 1925.

The stimulus for the production of antibodies is not simple but consists of different partial stimuli. These partial stimuli or partigens give rise to partial antibodies. A pure antigen is not a complete antigen. The difficulty of producing antibodies against pure proteins is just as great as that for pure lipoids. The purification of lipoids involves a denaturation. The pure lipoid, if it can be obtained in its genuine form, may be as antigenic as it is when admixed with protein in its natural source. While antilipoids may be produced

against lipoids free from any admixture, production is more successful when the lipoids are mixed with proteins of high molecular weight. Antilipoid formation is hindered when the lipoid is admixed with proteolytic degradation products. Antibodies are not produced for pure, neutral fats, except for triglycerides of fatty acids of high molecular weight, and only when these are mixed with protein or result from the activity of a fat-containing microorganism.

ARTHUR LOCKE.

EXPERIMENTAL BASIS OF ANTIBODY FORMATION AGAINST HOMOLOGOUS LIPOID. A. J. WEIL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **46**:84, 1926.

It is possible to produce experimentally a change in the blood that corresponds in all respects to the characteristics of syphilitic blood. This may be accomplished by injections into rabbits of lipoid extracts of homologous organs combined with foreign serum.

INVESTIGATIONS OF IMMUNITY IN ROCKY MOUNTAIN SPOTTED FEVER. F. BRIENL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **46**:123, 1926.

The observation by Ricketts and others that active immunity may be produced in guinea-pigs by simultaneous injection into several places of immune serum and living virus is confirmed. This immunity results from the survival for several days of the virus in the spleen.

A NEW IMMUNE PHENOMENON AGAINST SPIROCHAETA ICTEROGENES. I. L. KRITSCHEWSKY and R. S. TSCHERIKOWER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **46**:207, 1926.

In immunity against trypanosomes a thermostabile immune body is formed that is adsorbed by the parasites which then become covered with blood platelets in the presence of complement (H. Rieckenberg, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **26**:53, 1917). Kritschewsky and Tscherikower called this body thrombocytobarine, from thrombocyte and the Greek verb "barino," I load (ibid. **42**:131, 1925). They showed that a similar immune body develops in experimental recurrent fever caused by *Spirochaeta duttoni,* and now they describe specific thrombocytobarine in white rats and mice for *Spirochaeta icterogenes.*

ON THE INFLUENCE OF MANGANESE ON ANTIBODY PRODUCTION IN EXPERIMENTAL TYPHUS FEVER. E. SINGER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **46**:288, 1926.

The injection of manganese chloride in guinea-pigs infected with typhus fever hastens the production of agglutinins against OX 19. The serum of such animals also has higher protective value than the serum of central animals.

CONCERNING IMPEDIN-PHENOMENA IN PHAGOCYTOSIS. EXPERIMENTAL BASIS FOR DETERMINING AVIDITY AND TOXICITY OF ANTIGENIC MATERIAL. H. SUGURO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **46**:399, 1926.

Torikata (Koktopräcipitogene und Koktoimmunogene, Bern, 1917) found that native culture filtrates were less active in the precipitin reaction than boiled filtrates. He assumed the presence of a substance, which he called "impedin," that hinders the union of precipitinogen and precipitin. Suguro finds that a similar phenomenon can be demonstrated in other immune reactions, notably in phagocytosis.

REACTION TO LARGE DOSES OF DICK TOXIN. E. H. PLACE and A. BOURCART, Schweiz. med. Wchnschr. **56**:90, 1926.

Place and Bourcart injected by mistake 6,000 skin doses of Dick's toxin into two patients. The injection was followed by moderate general disturbances and a typical scarlatinal rash, with tendency to petechiae, followed by desquamation. They conclude that Dick's toxin is identical with the scarlet fever toxin.

RELATIONSHIP BETWEEN PROTEINS OF VARIOUS ORGANS. K. SAKURABAYASHI, Tohoku J. Exper. Med. **6**:427, 1925.

By means of precipitin reaction results were obtained indicating that the liver and the kidney of the dog contain common antigenic substance while the lung and the spleen seem to be distinct from each other as well as from the other organs.

Tumors

CHLOROMA: THE RECENT LITERATURE AND A CASE REPORT. DORSEY BRANNAN, Bull. Johns Hopkins Hosp. **38**:189, 1926.

Chloroma or chloroleukemia is a myelogenous process — an unusual form of myelogenous leukemia. Aleukemic stages of chloroma are common, whereas proved, true aleukemic forms of the disease are rare. Transitional or borderline and atypical cases occur, which emphasize the close relation between myelogenous leukemia and chloroma.

A classic case of chloroma is described.

AUTHOR'S SUMMARY.

CANCER OF UMBILICUS SECONDARY TO CANCER OF CECUM. JEROME R. HEAD, Surg. Gynec. Obst. **42**:356 (March) 1926.

A case is reported of cancer of the umbilicus secondary to adenocarcinoma of the cecum, which is rare in a woman 65 years of age. She had had symptoms of abdominal pain, vomiting and constipation for two years. A clinical and pathologic summary is presented of 101 cases of carcinoma of the umbilicus which have appeared in the literature up to the present time. Of these, three were primary squamous cell epitheliomas, twenty-two primary adenocarcinomas, and the remainder secondary to primary growths in the stomach, gallbladder, intestines, ovary and uterus, and of undetermined origin. Of eight cases secondary to cancer of the bowel, the primary lesion was located as follows: rectum, 3; transverse colon, 3; nearly all of large intestine, 1; cecum, 1.

M. L. PARKER.

REGISTRY OF BONE SARCOMA. E. A. CODMAN, Surg. Gynec. Obst., **42**:381 (March) 1926.

Five years ago the registry of bone sarcoma began with the object to keep an up-to-date list of living persons who had had bone sarcoma and who could be considered cured. At the end of five years, only seventeen cases of primary malignant bone tumors have been collected in which the patients can be considered cured. Of these, four are cases of Ewing's tumor and thirteen are osteogenic sarcomas. A complete report of patients cured of osteogenic sar-

comas is given with criteria for diagnosis in the history, physical, roentgen-ray and microscopic observations. The treatment used in these cases included amputation in all but one of the seventeen, mixed toxins in nine, radiation in eight and eight had all these combined. Seven had received no other treatment except amputation.

M. L. PARKER.

THE RÔLE OF TRAUMA IN DEVELOPMENT OF LATENT METASTATIC CARCINOMA. J. FIRKET, Ann. de méd. lég. **5**:374, 1925.

Four years after operative removal of an extensive carcinoma of the rectum which was apparently successful and followed by no recurrence, a secondary tumor developed in one foot two months after the foot was bruised by a heavy earthern pot which fell on it. During the two months after the injury of the foot intervening before a growth was noticed, the foot was never free from pain, and the growth was first noticed at the side of bruising. There was considerable surprise when the growth of the foot supposed to be either tuberculosis or sarcoma was found to be a cylindrical cell carcinoma with all the peculiarities of carcinomas of the rectum.

E. R. LECOUNT.

Medicolegal Pathology

BISMUTH POISONING FOLLOWING ORAL ADMINISTRATION OF BISMUTH SUBNITRATE. WILLIAM H. RESNIK, Bull. Johns Hopkins Hosp. **38**:333, 1926.

A case is reported in which bismuth poisoning followed administration of bismuth subnitrate by mouth. The important symptoms were a bluish-black discoloration of the gums, tongue and buccal mucosa, with slight ulceration, moderate anemia, basophilic stippling of the red blood cells, with tenderness and swelling of the paratid glands. There were also abdominal colic and evidences of a mild peripheral neuritis, but the dependence of these symptoms on bismuth poisoning is questionable. The patient recovered.

AUTHOR'S SUMMARY.

THE THEORY AND PRACTICE OF BLOOD GROUPING IN CASES OF PATERNITY. F. SCHIFF, Deutsche Ztschr. f. d. ges. gerichtl. Med. **7**:360, 1926.

After a discussion of the fundamental principles of Landsteiner's blood grouping and their application to the practical question of paternity in a given case, several cases were detailed in which the test was applied for legal purposes in matters involving the question of paternity. It is stated that this is the first time this test has been used in court for such purpose. The author believes that the test will be found to be of definite value in its proper field.

THE MEDICOLEGAL SIGNIFICANCE OF SUBARACHNOID HEMORRHAGE. WILLY MUNCK. Hospitalstid. **69**:149, 1926.

Nine cases of isolated subarachnoid hemorrhage causing sudden death are described. In such cases it is often highly difficult to decide whether the hemorrhage is traumatic or spontaneous because the pathologic-anatomic appearances may be the same in the two cases. It is necessary to have definite information

in regard to the history, and eventually also signs of external lesions in order to be sure that the hemorrhage is traumatic in origin. In most cases it is impossible to establish the point of origin of the bleeding.

THE MEDICOLEGAL INTERPRETATION OF INTRACRANIAL HEMORRHAGES FOLLOWING BLUNT FORCE APPLIED TO HEAD IN ADULTS. KNUD SAND, Ugesk. f. Laeger **88**:123, 1926.

Blunt force of various kinds and degrees applied to the head in adults may cause different forms of intracranial hemorrhages with or without external or internal lesions of obviously traumatic nature. Traumatic changes, when present, of course, are of the greatest value in determining whether the hemorrhage is traumatic in origin. Intracranial hemorrhages are of great medicolegal importance because they may produce sudden death, they may result from criminal violence, and they often are involved in problems of insurance. The following forms of intracranial hemorrhages are discussed: (1) The extradural hemorrhages which result most frequently from rupture of the middle meningeal artery from trauma with or without fracture. It is recalled that comparatively slight violence, e. g., blows with the fist, may cause such rupture, and that there is an interval between the trauma and the development of definite symptoms. (2) Subdural and leptomeningeal hemorrhages. The subdural hemorrhages may be purely traumatic or they may result from chronic internal hemorrhagic pachymeningitis, which itself may be traumatic in origin; this is rare, however, and the pachymeningitis then is unilateral and on the same side as the trauma. In nontraumatic pachymeningitis, slight injury, even only excitement or a fall, may result in a fatal hemorrhage. Obviously, it may be exceedingly difficult to reach a satisfactory interpretation in such cases. This is true also in regard to leptomeningeal hemorrhages which usually are most marked at the base, and the genesis of which may be difficult to explain. Most frequently it concerns ruptures in the basal arteries or of pial veins. Such hemorrhages may cause sudden death by plugging the fourth ventricle. (Concerning ruptures of aneurisms at base of brain, see E. Wallesch, (*Virchows Arch. f. path. Anat.* **251**:107, 1924). Leptomeningeal hemorrhage may arise spontaneously and after slight injury in young persons as well as in the old. Sand cites the following cases: A man, aged 45, in a quarrel received a blow from the fist on the chin, and died within two minutes from an enormous leptomeningeal hemorrhage; a man, aged 20, who apparently died while defecating, presents exactly the same picture but without any indications of external violence at all. Death while boxing may result from leptomeningeal hemorrhage. Here again it may be exceedingly difficult to reach definite conclusions as to the real cause of death. Obviously, a most painstaking examination of the cerebral blood vessels, including the pial veins, must be made in all cases of fatal intracranial hemorrhage in which trauma is suspected to have played a part. (3) Intracerebral hemorrhages. While fatal intracerebral hemorrhages frequently occur when there can be no question but that trauma is excluded, they may occur under conditions suggesting that trauma may have played a part in the causation, and in such cases it may be impossible to reach a satisfactory conclusion. (4) Hemorrhages in brain tumors by coincidence may give rise to similar difficulties of interpretation as intracerebral hemorrhages. Sand emphasizes the rôle that alcoholism and epilepsy may play in intracranial hemorrhages with and without trauma; also that meningitis, cerebral abscess and other forms of infection as well as epilepsy, cysts, etc., may follow such hemorrhages.

Technical

EXPERIMENTS ON THE SENSITIVITY OF THE HUMAN SKIN TO THE TOXIN OF THE BACILLUS OF SHIGA-KRUSE. H. BROKMAN and F. PRZESMYCKI, J. Immunol. **11**:361, 1926.

Experiments are presented showing that the negative skin reaction to the products of the Shiga-Kruse bacillus in human beings depends on the presence of antitoxin in the blood. The antitoxin is specific, and, as in the case of "natural" immunity to diphtheria, it appears spontaneously in the individual, without a previous attack of the disease or artificial immunization. If this presence of dysenteric antitoxin in human beings provides an actual immunity against infection with dysentery, then the way must be open to attempt an active immunity in the same way as is done in respect to diphtheria and scarlet fever.

AUTHORS' SUMMARY.

THE EFFECT OF HEAT AND HYDROGEN ION CONCENTRATIONS ON THE EXTRACT OF SHORT RAGWEED. L. N. GAY, J. Immunol. **11**:371, 1926.

From the three experiments with heat the author concludes that heat from even as short a period as one minute at 100 C. will reduce the activity of an extract of ragweed pollen. If the diluted solution is subjected to heat for increasing periods of time, the activity is reduced much more rapidly than if the concentrated solution is heated and then diluted. The violence of the reactions depends to a degree on the sensitiveness of the patient who is tested. If an extract of ragweed pollen is heated at 60 C. for one hour, and if various dilutions of the extract are then made, titration of it demonstrates a consistent reduction in the intradermal reactions. If this extract is treated in the same way after having been subjected to heat at 100 C. for one hour, the intradermal reactions are even less marked than in the first instance. The autoclaving of an extract reduces the active principle to such a degree that sufferers from mild attacks of hay-fever fail to give an intradermal reaction to such a solution; sufferers from severe attacks give a greatly diminished reaction. Alteration of hydrogen ion concentration in no way affects the intradermal reaction of unheated or of heated extracts.

AUTHOR'S SUMMARY.

A PLEA FOR A STANDARDIZED METHOD OF ESTIMATING AND REPORTING HEMOGLOBIN VALUES. J. W. LINDSAY, E. C. RICE and M. A. SELINGER, J. Lab. & Clin. Med. **11**:737, 1926.

Few hemoglobinometers on the market are sufficiently accurate for clinical work. Hematologists should make a practice of reporting the hemoglobin content of the blood in grams per hundred cubic centimeters as well as per cent. From 14 to 17 Gm. per hundred cubic centimeters is suggested as the normal limits of the hemoglobin content of the blood, these figures being used in the same manner as the normal in blood chemistry. Wong's method of determining hemoglobin is sufficiently accurate for standardization of hemoglobinometers in the clinical laboratory. The hemoglobinometer devised by Newcomer using a standardized glass color plate is recommended as the most accurate instrument for clinical work.

S. A. LEVINSON.

Society Transactions

MINNESOTA PATHOLOGICAL SOCIETY

Feb. 16, 1926

MOSES BARRON, M.D., *President*

ACUTE INTERSTITIAL NEPHRITIS WITH RENAL INSUFFICIENCY. JOHN F. NOBLE. (From the Department of Pathology, University of Minnesota and the Ancker Hospital Laboratory, St. Paul, Minn.)

Acute interstitial nephritis was first described by Biermer (*Virchows Arch. f. path. Anat.* **19**:537, 1860) as a complication of scarlet fever. Subsequently there were important contributions by Sorenson (*Ztschr. f. klin. Med.* **18**:298, 1890), Friedländer (*Fortschr. d. med.* **1**:81, 1883), Councilman (*J. Exper. Med.* **111**:393, 1898), Reichel (*Ztschr. f. Heilk.* **6**:72, 1905) and others. The contributions have dealt almost entirely with the disease from a pathologic standpoint, and little is known of the clinical features of this type of renal disease.

This condition is found most frequently associated with the acute infectious diseases of children, such as scarlet fever, diphtheria and measles. It is more rarely found in such conditions as cerebrospinal meningitis, lobar pneumonia and pericarditis. It has also been observed in severe cases of smallpox. Councilman reports the condition in a varying degree in practically 25 per cent of the cases in a series of 146 necropsies in scarlet fever, diphtheria and combined scarlet fever and diphtheria patients.

The kidneys, when examined grossly, are usually found to be larger than normal. They show a marked degree of cloudy swelling and resemble in appearance the kidneys in an acute nephrosis. Subcapsular hemorrhages are frequently found. The pelves of the organs are normal.

Microscopically, the kidneys show an inflammatory infiltration of the interstitial tissue which is usually focal in distribution, but sometimes occurs diffusely. When the focal arrangement of the inflammatory cells occurs the most frequent locations are "in the boundary zone of the pyramids, the subcapsular region of the cortex, or around the glomeruli." The presence of plasma cells in large numbers is characteristic of the exudate. The tubules in the infiltrated areas are often compressed by the interstitial exudate. Polymorphonuclear leukocytes are frequently found in the lumina of the tubules. There is no evidence of a glomerulitis, and there are no changes in the arterioles. The swelling and opacity of the cortex is due to the cloudy swelling of the tubules as well as to the interstitial infiltration.

Renal insufficiency in acute interstitial nephritis is apparently an infrequent occurrence. Biermer reports one of his three patients as having an oliguria and dropsy. This patient, he states, died of uremia. Sorenson also observed cases of this disease in which there was a slight edema and ascites present. Councilman, in his series, found that the cases invariably showed an albuminuria which was more marked than that frequently seen in cases of diphtheria without acute interstitial nephritis. He never observed diminution in the amount of urine or edema in any of his cases. Reichel states, however, that patients with acute interstitial nephritis may die of uremia. All of these reports were published before our newer methods of determining renal insufficiency were available, and it is likely that the condition was overlooked in many of the cases. Veeder and Johnston (*Am. J. Dis. Child.* **19**:223, 1920) have recently

reported a case of acute interstitial nephritis with a nonprotein nitrogen retention of 47 mg. per hundred cubic centimeters of blood and 30 per cent of phenolsulphonphthalein. In this case report no clinical symptoms of uremia are noted.

The following case is reported because of the severe degree of kidney involvement found at necropsy and because of the fact that the patient showed definite evidence of renal insufficiency.

A man, aged 23, was admitted to the Ancker Hospital, St. Paul, in the service of Dr. Critchfield, Dec. 19, 1925. On admission his chief complaints were sore throat, rash, headache and general weakness. The onset of his illness occurred on Dec. 12, 1925, when he first noticed a sore throat and a general feeling of malaise. He continued to work for five days after the onset of his illness, but was very miserable during this period. On the morning of December 19, a rash was noticed over the surface of his abdomen, and a physician was called who made a diagnosis of scarlet fever. On admission to the hospital, the patient had a temperature of 101.3 and a pulse rate of 90.

Physical examination showed the patient to be well developed and well nourished. Practically his entire body was covered by a diffuse punctate erythematous rash which was typically the rash of a scarlet fever. He had a strawberry tongue, and his tonsils, pharynx, uvula and soft palate were markedly inflamed. The tonsils showed a grayish-white exudate in certain areas, which was easily removed. The cervical lymph nodes were palpable. Examination of the lungs showed nothing of note. Examination of the heart showed no murmurs, and the rhythm was regular. Examination of the abdomen and extremities showed nothing of note.

At the time of admission the patient was not considered seriously ill, and his condition remained satisfactory until December 24, when his temperature suddenly dropped and he apparently went into a state of shock. After he recovered somewhat from this condition, 10 cc. of scarlet fever antitoxin was given. The patient showed no evidence of improvement, and on December 25, his blood pressure was 80 systolic and 43 diastolic. His condition remained about the same until December 27, when he showed slight improvement, being somewhat brighter mentally.

On the morning of December 29, he was unable to void, and this anuria continued until his death at 8:05 p. m. on December 30. The bladder was catheterized on December 29, but no urine was obtained. Convulsions, tremors and coma were not observed.

In reviewing the urinary observations in this case, it is interesting to note that the first urine, examined on December 21, was normal. Examination of the urine on December 24 and 26, showed a faint trace of albumin, but the microscopic examination was negative. It was not until December 28, that the urine showed a pathologic sediment, and at this time only a few hyaline casts were observed. A small specimen of urine was collected from the patient before 6:30 a. m., December 29. This specimen showed a three plus albumin, many hyaline casts, pus cells and red blood cells. The rapid increase in the quantity of casts and leukocytes fits into Reichel's idea that the disease may be rather far advanced before pathologic urine is observed.

A chemical test of the blood taken on the morning of December 29, shortly after the patient had developed an anuria and thirty-six hours before his death, showed 174.3 mg. of urea nitrogen and 10.6 mg. of creatinin per hundred cubic centimeters of blood.

At necropsy the body was found to be that of a well developed and well nourished white man. There was no evidence of edema, cyanosis or jaundice.

General examination of the abdomen and thoracic cavities revealed little of note. On opening the pericardial cavity, however, the visceral and parietal pericardium were found to be adherent. The surfaces were covered by a thick layer of yellow fibrin, and there was about 10 cc. of a purulent fluid present. Examination of the heart itself showed nothing of note except a marked cloudy swelling of the muscle. The valves were normal. There was a rather marked edema and congestion of the lungs, and there were small areas of consolidation scattered throughout the lower lobe of the right lung. The liver was unusually large, weighing 2,690 Gm. On cut section the organ showed a severe degree of cloudy swelling, but was otherwise normal in appearance. The spleen was enlarged, weighing 600 Gm. On cut section the pulp was found to be very soft and almost diffluent in character. The right kidney weighed 520 Gm. and the left 570 Gm. The capsules of the organs stripped very easily, leaving a smooth surface and the cut edges of the organs everted. There was no evidence of petechial hemorrhages on the surfaces of the organs. On cut section the cortices were found to be very wide, and the demarcation between the cortex and the medulla was well defined. The other markings of the organ were indistinct, and they were very pale and swollen in appearance. The pelves and ureters were not dilated, and their mucosal surfaces were smooth and showed no hemorrhages. The anatomic diagnosis was: acute interstitial nephritis (based on microscopic examination), acute pericarditis, acute splenitis, cloudy swelling of the heart and liver and early bronchopneumonia.

Microscopic sections of the kidneys showed a marked uniform infiltration of the interstitial tissues of the entire cortex and medulla. In no part of the kidneys was the exudate focal. The tubules were markedly compressed by the exudate; some were distinctly atrophied, and in large areas they had entirely disappeared. Apparently the pressure of the exudate caused the atrophy and disappearance of the tubules. Some of the tubules contained many polymorphonuclear leukocytes, and casts were frequently observed. Blood cells were occasionally found in the lumina of the tubules. The glomeruli showed no definite histologic changes, but in a few glomeruli the exudate was seen to break through Bowan's capsule into the glomerular spaces. The exudate consisted largely of plasma cells, polymorphonuclear leukocytes and large mononuclear cells. Many of the plasma cells showed two nuclei, and a few mitotic figures were observed. The gross and microscopic structure of the kidneys supported the chemical evidence of renal insufficiency.

Gram-Weigert stain of the kidneys showed definite clusters of gram-positive cocci in the interstitial tissue, occurring more frequently intracellularly, in clusters and pairs. No further studies were made to determine whether these cocci were the cause of the inflammatory exudate. Councilman found a variety of organisms in his cases, but thought that they had no relationship to the disease.

The case was one of acute interstitial nephritis with gross, histologic and chemical evidence of renal insufficiency.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS IN RABBITS. B. J. CLAWSON.

Plain agar was heavily seeded with *Streptococcus viridans* and incubated at 37 C. for twenty-four hours. The agar with the organisms was ground in a mortar with salt solution until a fine suspension was formed. The coarser particles of agar were then thrown out of the suspension by centrifugalization. The suspension was then injected into the left ventricle of rabbits. Repeated injections were made at intervals of about ten days. The small emboli lodged

in the glomerular capillaries and produced focal embolic glomerulitis closely resembling the renal lesions associated with subacute bacterial endocarditis. (This paper appears in full in the ARCHIVES OF PATHOLOGY AND LABORATORY MEDICINE **1**:911 [June] 1926.)

TWO RARE CASES OF HODGKIN'S DISEASE: (A) CHRONIC RELAPSING FEVER OF PEL-EBSTEIN TYPE; (B) HODGKIN'S DISEASE WITH EXTENSIVE CUTANEOUS MANIFESTATIONS. MOSES BARRON.

A man, aged 42, in April, 1925, became ill with a high fever which lasted several days. Regularly every fourth Saturday after this he had a similar attack. The temperature would go as high as 104 or 105, with a pulse rate from 120 to 140, and respirations 30 to 40. After a few days he would slowly recover. He was admitted to St. Mary's Hospital on Jan. 5, 1926, during one of these febrile attacks. The fever persisted until death. Physical examination revealed a number of enlarged cervical lymph nodes. The spleen was palpable. No other important observations were made. Urinalysis showed albumin +, no sugar and many granular casts. Chemical test of the blood on January 5 revealed: erythrocytes, 5,000,000; hemoglobin, 85 per cent; leukocytes, 4,100 to 4,900; polymorphonuclears, 59 per cent; lymphocytes, 38 per cent. The Wassermann reaction was negative. During the last two or three days an intense jaundice developed. Two cervical lymph nodes were removed for examination. These consisted almost entirely of necrotic tissue with a thin peripheral zone of fibrous tissue. The node was not recognized as Hodgkin's disease. The patient died on Jan. 26, 1926. The spleen weighed 900 Gm. and showed the characteristic gross and microscopic features of Hodgkin's disease. Small nodules of Hodgkin's disease were found throughout the liver. There was marked involvement of the lymph nodes along the aorta, in the mediastinum and in the cervical region. A striking feature was the extensive area of necrosis in these nodes, but the typical Hodgkin's tissue was also present.

The second case showed extensive cutaneous lesions.

THE RELATION OF ACHLORHYDRIA AND INTESTINAL FLORA TO PERNICIOUS ANEMIA. J. B. CAREY.

Dr. Carey studied the gastric and duodenal contents in pernicious anemia for the colon bacillus. He concluded that there was no satisfactory evidence that the colon bacillus was the cause of this disease.

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, May 10, 1926

RUTH TUNNICLIFF, M.D., *President, in the Chair*

SKIN REACTIONS WITH THE FILTRATE OF A KOCH STRAIN OF B. TUBERCULOSIS CULTIVATED ON A SYNTHETIC MEDIUM. DR. RUSSELL D. HERROLD and DR. CLARENCE C. SAELHOF. (John McCormick Institute for Infectious Diseases, Chicago.)

The so-called Koch strain of the tubercle bacillus is an avirulent culture which grows profusely on the ordinary mediums. A substance may be formed in a synthetic medium which gives a skin reaction. Results with nutrient broth containing 0.1 per cent of dextrose and 0.1 per cent of dibasic sodium

phosphate in place of sodium chloride were reported recently (J. A. M. A. **86**:747, 1926). Dr. Novy kindly supplied the culture. It is the strain brought from Dr. Koch's laboratory in 1888, and originally was virulent.

Cultures were made on Long's synthetic medium. After five days the medium was passed through Berkefeld N filters, and the filtrate was diluted with salt solution (0.9 per cent) for the skin tests. One tenth cubic centimeter intracutaneously produced skin reactions in normal persons with dilutions up to 1:50.

Most normal persons in twenty-four hours had redness at the site of injection from 1 to 3 cm. in diameter, the maximum being reached in from twenty-four to forty-eight hours. Often a pigmented spot persisted for several days and sometimes for several weeks. Such reactions were arbitrarily classified as positive, and in degree are one, two, and three plus. Doubtful reactions are from 5 to 10 mm. in diameter, and after forty-eight hours have disappeared. Controls of medium diluted with salt solution were negative.

At present seventeen frank cases of pulmonary tuberculosis, confirmed by stained preparations, have been tested. Of these, sixteen were negative and one slightly positive, as was the control. Subsequent retesting of this person had the same result even with 1:25 and 1:10 dilutions of toxin.

In a series of 33 persons with no clinical tuberculosis, the following results were obtained:

Toxin	Negative	Plus-minus	1 plus	2 plus	3 plus	Control
5 day filtrate 1:50....	1*	1†	11	15	5	neg.

* Underweight, temp. 97 C.
† Temperature 100 in twenty-four hours.

Results with filtrates of five and seven days cultures in eleven consecutive cases were:

7 day 1:100...	+	—	—	±	++	—	—	±	+++	+++	++
5 day 1:50....	++	+	±	+†	++	+†	+†	+†	+++*	+++	++

* Markedly pigmented for two weeks.
† Colored persons.

This test was applied to fourteen students, apparently normal. Of these, four had two and three plus readings, and some roentgen-ray evidence of calcified tuberculosis was found. The ten others had lesions apparently with no calcification, and their reactions were negative. It seems that with healing, less antisubstance is produced by the tubercle to neutralize the injected toxin. However, this series is too small for definite conclusions. The interpretation of these results is tentative. The presence of a reaction in the majority of apparently normal adults seems to indicate that a substance is produced by the growth of this strain of tubercle bacillus in a synthetic medium which acts in a different manner than tuberculin. This is in accord with results reported recently.

Further tests on a large number of patients are indicated with filtrates from a variety of strains of tubercle bacilli. The reaction to the filtrate in normal adults and the absence of a reaction in tuberculous patients may mean that with an active lesion there is sufficient antisubstance to neutralize antigen According to results with the fourteen patients examined by roentgen ray, apparently there is a relationship between intensity of skin reaction and calcium deposition or healing, because the more severe the reaction, the greater is the calcification and inactivity of the tuberculous lesion.

AN ANOMALOUS BAND ACROSS THE CHANNEL OF THE AORTA AT THE LEVEL OF THE AORTIC LEAFLETS. SEYMOUR WEINSTEIN. (From the Pathological Laboratory, Rush Medical College, Chicago.)

In 1878, Archer (*Dublin J. M. Sc.* **65**:405), 1878) reported a congenital band which stretched loosely across the lumen of the aorta just above the insertion of the leaflets. It arose by an expansion 1.25 cm. wide just above the junction of the posterior and left lateral semilunar leaflets and gradually narrowed becoming inserted at the junction of the posterior and right lateral leaflets. It seemed to consist of the same kind of tissue as the leaflets.

Archer believed that the band developed with the semilunar leaflets, and was to be regarded as an irregular and supernumerary cusp.

In 1896, Röhrle (*Deutsche med. Wchnschr.* **22**:270, 1896) described a more tendinous band in the aorta of a child as the only anomaly of that sort found in 25,000 necropsies at the Foundling's Home in Moscow.

One end was at the left end of the left semilunar cusp, and it stretched tightly across to the left end of the right semilunar cusp. Its ends were thin like threads, and at its center it was 5 mm. in diameter.

In 1900 Hektoen (*Tr. Chicago Path. Soc.* **3**:105, 1900) reported an observation he made of a large defect at the base of one of the aortic cusps, which he ascribed to a defect in the development of the aortic septum. At this time he cited several instances of anomalous cords at the level of the aortic cusps.

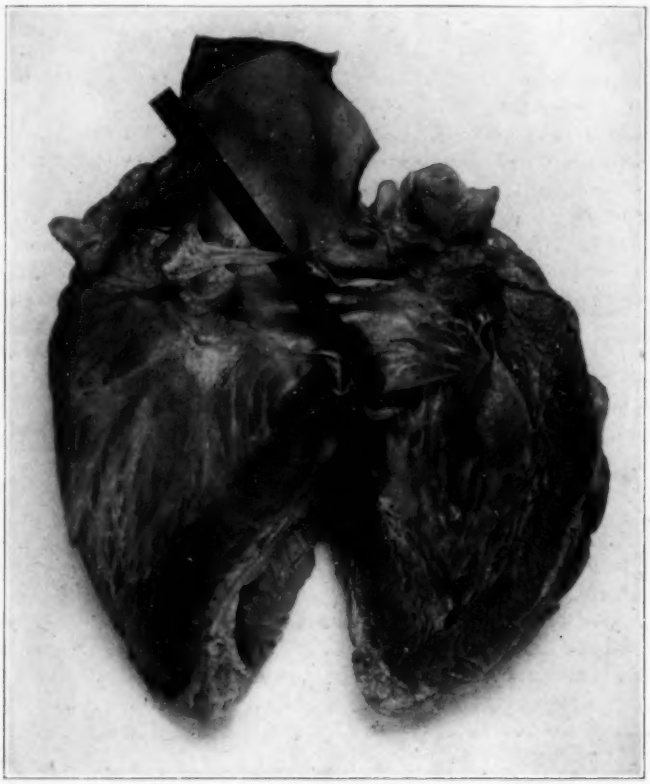
Closely related to these is the condition in a heart from the body of a negro, aged 50, admitted to the Cook County Hospital to the service of Dr. Loeb with a clinical diagnosis of cirrhosis of the liver and ascites. The examination revealed little except the ascites. The heart was normal in its rate, rhythm and sounds; there was no murmur.

He died nine days after entrance from chronic incomplete intestinal obstruction complicated by empyema. (Necropsy by Dr. Singer, resident pathologist.) The heart weighed 202 Gm. The epicardium over the left ventricle was gray and opaque. There was considerable subepicardial fat. The right ventricle was soft and almost completely covered with fat; the left was firm. The surfaces made by cutting the left myocardium were smooth, red, moist and glistening, and the margins were infiltrated with fat. The myocardium of the left ventricle measured 10 mm. at the base, 7 at its middle and 6 at the apex. The surfaces made by cutting the myocardium of the right ventricle were smooth, brown-red, moist and glistening, and the infiltration of fat was greater than in the left. The myocardium was 3 mm. at its base and 2 mm. at the apex. The left ventricular endocardium was smooth, moist and glistening, and the papillary muscles and chordae tendinae were normal. The right endocardium was covered by fatty tissue, and there was a bit of fat about the papillary muscles and chordae tendinae.

The mitral valve ring was 8 cm. in circumference. The posterior leaflet was unchanged. The anterior leaflet was wrinkled, moist and glistening and its free margin was somewhat thickened and firm. The aortic ring was 5 cm. in circumference. The cusps were normal. A triangular cord crossed the aorta just distal to the leaflets. One end 1.5 cm. wide was attached to the aorta just beyond the right cusp, the other much more tenuous end was attached to the aortic walls just above the common insertion of the posterior and left cusps. The tissue of the cord appeared similar to that of the cusps. The pulmonic ring was 6 cm. in circumference; the leaflets were normal.

The interventricular septum was normal. The aorta was dilated at its base. The lining was smooth and moist. There was a small yellow patch in the intima 8 by 2 mm. just distal to the mouth of the posterior coronary artery with the intima slightly puckered there. The pulmonary artery was elastic and its lining was smooth, moist and glistening. The walls of the coronary arteries were rather hard, the channels tortuous. Their lining was smooth. The auricles were normally smooth.

Abbott (Osler and McCrae: Modern Medicine, Philadelphia, J. B. Lippincot Company) groups cardiac anomalies thus: (*a*) those due to the arrest of



Heart from a negro, aged 50, showing anomalous band crossing the channel of the aorta just distant to the aortic leaflets; a marker passes under the band.

growth at an early stage before the different parts of the heart have been entirely formed, and (*b*) those produced at a later stage by fetal diseases.

The normal appearance of the aorta with this band near its root, together with the normally formed aortic cusps, indicates that the condition in all probability did not arise because of some disease of the fetus in utero, but with the development of the semilunar cusps.

The exact development of the semilunar cusps is not definitely settled to date. It is the present day notion (Tandler, J.: The Development of the Heart, Philadelphia, Keibel and Mall, **2**:534-569, 1912. Humphrey, L.: Congenital Malformations of the Heart, in Allbutt: System of Medicine, London,

6:697-725, 1898. Rokitansky, C. F.: Die Defekte der Scheidewände des Herzens, Wien., 1875) that they are hollowed out from endocardial thickenings in the transitory bulbus aorta of the embryonic heart.

In this heart it is possible that supernumerary endocardial thickenings formed distal to those from which the aortic leaflets developed and by growing came into contact so as to form a cord instead of hollowing out into accessory cusps.

A Congenital Perforate Interventricular Septum of the Heart Accompanied by a Shortened Medial Tricuspid Leaflet and a Dilated Pulmonary Artery with Two Cusps. Seymour Weinstein. (From the Pathological Laboratory, Rush Medical College, Chicago.)

In 1922, Gutzeit (*Virchows Arch. f. path. Anat.* **237**:355-372 [Nov. 22] 1921) described a congenital opening in the membranous portion of the interventricular septum with defects of the tricuspid leaflets. The septal opening was 16 by 11 mm., situated under the posterior and partly under the right aortic cusps. Its margins were fibrous tissue. On the right side it was covered by the combined insertion of the medial and posterior tricuspid leaflets, the medial firmly attached to the fibrous margin of the opening in front and below and the posterior similarly bound behind and below along the margin of the opening in the septum. The upper posterior margin of the opening had no connection with the tricuspid leaflets. Also the medial tricuspid leaflet was narrow and thick, measuring 14 mm. at its distal margin and 13 at its middle and insertion, and at its insertion it had a plexiform trabeculated arrangement. Lying between two trabecular thickenings there was an oval opening, 16 by 3 mm., with a sharply defined margin immediately under the posterior insertion of the medial leaflet exactly opposite the ventricular septum defect.

These leaflets were attached above the annulus fibrosus and formed with the posterior tendinous membrane, which demarcated the septal defect, a pocket 8 mm. deep into which a sound could easily be passed through the defect into the left ventricle.

One of the hearts I have examined is quite like that described by Gutzeit. It is the heart of a child 6 months old admitted to the service of Dr. Bachmann and Dr. Gibson at St. Luke's Hospital, Chicago, with a clinical diagnosis of bronchopneumonia. Physical examination of the chest failed to disclose anything of interest with reference to the heart. The child died the day after entrance and the necropsy and histologic examinations by Dr. Edwin F. Hirsch confirmed the clinical diagnosis.

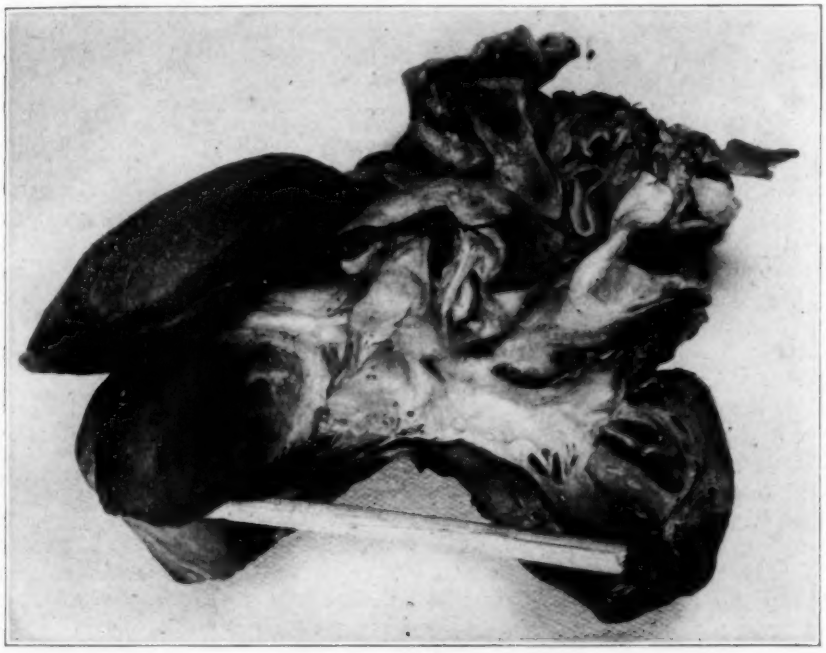
The heart weighed 53 Gm. The endocardium, epicardium and surfaces made by cutting were smooth and normal. The myocardium of the left ventricle measured 7 mm. at the apex and middle and 8 at the base. The anterior wall was markedly trabeculated, and the papillary muscles were heavy. The chordae tendinae were normal. The mitral leaflets were smooth and somewhat narrow. The mitral ring was 4.5 cm. in circumference, the aortic 3. The aortic cusps and lining were smooth and normal.

The right ventricle was markedly trabeculated. The tricuspid ring was 5 cm. in circumference. The myocardium of the right ventricle was 1 mm. at the apex, 5 at its middle and 3 at the base. The posterior and anterior tricuspid leaflets were smooth and normal. The papillary muscle of the anterior leaflet was thick and the chordae tendinae were well developed. The medial leaflet was 8 mm. at its origin and 10 mm. at its free margin. It was brought up somewhat tightly against the defect in the upper part of the

interventricular septum. It was trabeculated distally, and between two thickened fibers on its posterior surface there was a valve opening 2 mm. in diameter. This was directed downward toward the right ventricle. The papillary muscle of this leaflet was rather ill defined and fused with the septum. The chordae tendinae were few and threadlike.

The septum had an opening 8 mm. in diameter below the right and middle aortic cusps, and directed downward and posteriorly into the right ventricle, with a well defined margin of fibrous tissue.

The pulmonic ring was 3.2 cm. in circumference. The pulmonary artery just above the valve was dilated to 3.8 cm. in circumference. Its intima was smooth. The valve had only two cusps, the right much the larger. Both were well formed.



Heart of a 6 months old child showing shortened medial leaflet of the tricuspid valve against the interventricular septum covering a defect in the septum. It may be noted that the papillary muscle of this leaflet is not well developed; also the thickened papillary muscle of the anterior leaflet.

The right auricle was moderately deepened and trabeculated. The left auricle was thin-walled and more or less smooth.

The foramen ovale was present as an oblique opening.

Congenital defects of the tricuspid leaflets are rare, and only a few have been reported. The first report, according to Gutzeit, was that by Reick in which the defect in the tricuspid was so great that the auricle was separated from the ventricle only by a ring-shaped swelling. He also mentions the hearts reported by Ebstein in 1866 in which a sack was suspended into the right ventricle from the cartilaginous bicuspid ring. This had only a lateral insertion in the annulus and went over medially into the endocardium of

the septum. The hypertrophied front and back leaflets hung in the sack, and there was only a remnant of the middle. In other reports by Marxsen, MacCallum, Geipel and Schoenenberger the medial leaflet was underdeveloped. In the three hearts described by Heigel the anterior leaflet was most developed and the medial least. The conditions in all these hearts, the anomalies of the tricuspid leaflets, have been due to maldevelopment.

The accounts by Preis and Hart deal with two hearts, each with a perforate interventricular septum and defective development of only the front tricuspid leaflet. In that described by Preis the right ventricular cavity was moderately enlarged; the septal opening was 10 by 12 mm. and bounded below by the septum, above by portions of the back and right aortic leaflets and in front by the aortic septum. The auricular septum failed to meet the ventricular septum behind. This defect reached backward and considerably above the aortic opening up to the middle of the auricular septum. The pars membranacea was absent. The anterior leaflet of the tricuspid valve was considerably lengthened, and the medial leaflet was thickened. The contiguous halves of these leaflets were brought up against the defect by a strong thick band which fixed them against the right margin of the opening.

In Hart's report, the heart was also that of a child who died from pneumonia when 1 year old. The left ventricle was slightly dilated. The great vessels were normal. Immediately under the right aortic cusp there was an oval opening with a slightly thickened margin bounded above by the insertion of the aortic cusps. By transillumination the tissue close about the opening, especially the posterior aortic cusp, was translucent. The defect was in the wall of the right ventricle where the anterior and medial tricuspid leaflets joined. The medial leaflet possessed old knotty thickenings the size of a pinpoint, and there was marked contraction of the chordae tendinae.

It has not been decided how the shortening of the medial tricuspid leaflet occurs, but the development of these leaflets is closely associated with that of the septum between the ventricles.

The internal division of the heart into right and left sides is affected by three septums. These appear within the cavity of the heart perfectly independent of one another. They are the auricular, ventricular and truncus arteriosus septums. The greater part of the ventricular septum is formed from the septum inferius. It is completed above partly by the endocardial cushions at the lower edge of the interauricular septum, the intermediate septum of His and by the prolongation of the aortic septum. The aortic septum grows caudad beyond the truncus arteriosus so as to project a certain distance into the ventricular cavity. It then fuses with the free lower edge of the interauricular septum, while finally the septum inferius extends so as to meet and fuse with the interauricular septum.

In these malformations the membranous septum is wanting and the septum inferius fails to reach and fuse with the septum superius. The presence of other defects and the absence of any evidence of inflammation point definitely to some interference with the normal processes of growth in embryonic life. A bicuspid pulmonic valve is not uncommon, is frequently associated with defects of the interventricular septum and is probably due to a suppression of one of the endocardial cushions. The slight hypertrophy of the wall of the right ventricle is due to the forcing of blood through the opening in the septum into the right ventricle with each systole and the increased work thus thrown on that part of the heart. The anomaly of the medial tricuspid leaflet was probably associated with a leakage, failure of the leaflets to close

the tricuspid opening, and brought about the dilatation of the right auricle. An increased volume of blood sent into the pulmonic artery probably caused dilatation of that vessel.

HEALING SPONTANEOUS RUPTURE OF THE POSTERIOR MITRAL PAPILLARY MUSCLE. F. K. POWER. (From the Henry Baird Favill Laboratory of St. Luke's Hospital.)

Spontaneous rupture of a papillary muscle of the heart, according to reports in the literature, is rare. The first account seems to have been made by Courvisart (Diseases of the Heart [transl. by Jacob Gates], 1812, p. 199) who records this disorder in the heart of an adult dying suddenly, and the rupture of a mitral valve papillary muscle was caused by a suppurative inflammation. Bertin (Traite des maladies du coeur et des gros vaisseaux, Paris, 1824, p. 52) reports the traumatic rupture of a papillary muscle which was thought to have resulted from the stress of coughing, although there were vegetations on the chordea tendinae. In a report by Dennig (*Deutsches Arch. f. klin. Med.* **96**:163, 1909), a similar rupture followed severe illness with a thrombosis of the coronary arteries. The report by Heiman (Laennec, R. T. H.: Diseases of the Chest [transl. by John Forbes], 1838, p. 718) is unusual in that a papillary muscle of the tricuspid valve was torn, and he lists an account by Legendre in which a severe blow on the chest caused the rupture of a mitral valve papillary muscle. Wankel (Ein Fall von spontaner Papillarmuskel zerreissung, I. D. Giessen, 1911) in 1911 described a rupture of the anterior muscle of the mitral valve due to multiple thrombi of the coronary arteries, especially the ramus verticalis of the descending branch of the left coronary artery. Microscopically, there was a necrosis of the papillary muscle stump and a marked fatty degeneration of the myocardium.

Teacher (*Glasgow M. J.* **75**:374 to 376, 1911) describes the heart of a man, aged 64, whose death occurred suddenly, and postmortem examination demonstrated a left mitral papillary muscle torn off in the middle portion. The edges were frayed and the muscle infiltrated with blood for a short distance on either side of the tear. The edges of the torn surface were curled slightly. In sections of the ruptured end of the papillary muscle, the distal portion was covered with a white thrombus containing a few red blood cells and many leukocytes. There was a marked infiltration with polymorphonuclear leukocytes between the ruptured fibers. Close to the rupture, the fibers were in various stages of degeneration, the cells shrunken, the striations lost and the nuclei absent.

In Spaulding's report (*Bull. Johns Hopkins Hosp.* **32**:30, 1921) of sudden death in a man, aged 31, the heart at the postmortem examination weighed 560 Gm., and the posterior (inferior) papillary muscle of the mitral valve, which was "Y" shaped, was torn in the inferior pillar, close to where the chordae tendinae were attached. The fragment attached to the valve was yellow, the endocardium smooth but opaque. The torn end was rough, irregularly conical in shape, and covered with a firm adherent clot. The center contained muscle which was surrounded by a yellow ring of necrotic tissue just beneath the endocardium. The stump was covered by endocardium. The line of demarcation was sharp and bordered by a zone of hemorrhage. There were petechial hemorrhages of the other papillary muscles. Microscopically the central part of the papillary muscle consisted of necrotic tissue with nuclear fragments, and along the margin of this necrotic region, many leukocytes. Beyond the leukocyte zone the muscle fibers were shrunken, the striae and

nuclei absent, and there were small hemorrhages between the atrophied fibers. The muscle cells adjacent contained fat droplets. In Levaditi preparations spirochetes were found just beyond the necrotic region, but the tissue change did not resemble a gumma.

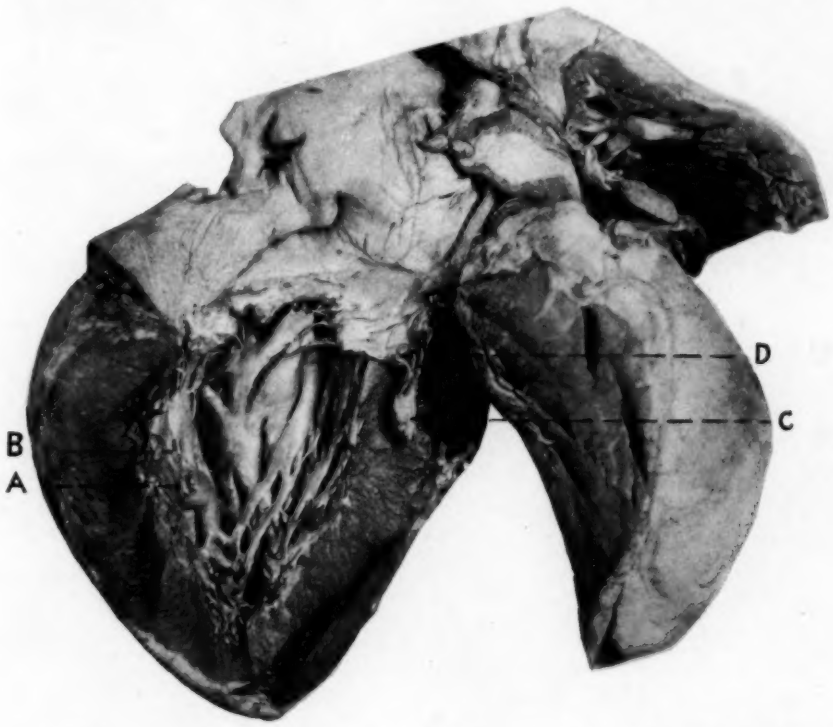
Of these reports, that by Teacher is the only one in which healing of the torn fragment of papillary muscle is recorded, and the healing process in this is presumed to be of not more than a week's duration. The surface of the torn papillary muscle in the heart described in the following report has healed completely.

A. R., a negress, aged 42, was admitted to St. Luke's Hospital, Dec. 3, 1925, to the service of Dr. Robert Preble, complaining of dyspnea, slight edema of the feet and precordial pain for three months. When admitted there was no generalized edema, but the liver was four fingerbreadths below the costal margin, the heart much enlarged, with a systolic murmur heard best over the apex and transmitted to the axilla, appearing to be an old mitral lesion with decompensation. The patient became gradually worse, the dyspnea increased, the extremities became edematous and the edge of the liver descended five fingerbreadths below the costal margin. The temperature recorded was about 100 F. On Jan. 3, 1926, the patient reported a severe heart attack the night before, with pain in the epigastric region and extreme dyspnea. The heart rate was 105 and very irregular. Morphine gave some relief. The following morning she was resting more comfortably. On January 7, there was some fluid in the right side of the chest, and her general condition seemed better. Following this she gradually became worse, dyspnea increased, and the pulse became irregular. At 3 a. m. on Jan. 12, 1926, the pulse was markedly irregular, the rate 96, respirations 18. At 5:36 the pulse could not be obtained at the wrist, and death followed shortly.

The Wassermann test of the blood was negative. The blood contained 29.4 mg. of urea nitrogen, 56 mg. of nonprotein nitrogen per hundred cubic centimeters. The urine contained a trace of albumin. No bacteria were found by cultures of the blood.

Necropsy was performed by Dr. Edwin F. Hirsch. The essential changes concerned the heart and those associated with cardiac decompensation. The lower margin of the right lobe of the liver was 4 cm. below the costal arch. There was marked emphysema of the lungs. The amount of pericardial fluid was slightly increased. The heart was 17 cm. obliquely and 12 cm. transversely. The tricuspid ring admitted the tips of five fingers, the mitral of four fingers. There was marked hypertrophy of the myocardium of the left ventricle; its maximum thickness, midway between the apex and the mitral ring was 3.5 cm. The heart with 40 cm. of the aorta and 3 cm. of the pulmonary vessels weighed 890 Gm. There was a recent embolus occluding the right branch of the pulmonary artery. On the posterior wall of the left ventricle, 5 cm. from the apex of the heart, was a ruptured posterior papillary muscle (*A* in illustration). In this place there was a conical stump, 1.1 cm. in diameter, projecting 1 cm. into the lumen of the ventricle. The tip of the muscle stump contained a circular depression about 7 mm. in diameter and about 1 mm. deep. The center of this region was elevated red-brown tissue, the peripheral edges of which were curled inward overlapping a conical elevation 2 mm. in diameter, and a similar whiter elevation 1 mm. in diameter. On the superior surface of the apex of this fragment of muscle was a tag of white tissue 2 mm. long and 0.5 mm. in diameter. The surface of this fragment of papillary muscle was covered with a smooth, shiny endocardium. Opposite this papillary muscle

and attached to the posterior leaflet of the mitral valve by three chordae tendinae, which averaged from 1.3 to 1.8 cm. in length and from 0.5 to 1 mm. in diameter, was another fragment of papillary muscle which seemed to have been torn from the stump described above (*C* in illustration). This fragment was 1.2 cm. long and from 0.3 to 0.5 cm. in its other dimensions. From 4 to 6 mm. of the surface of this fragment was covered by gray, shiny tissue-like endocardium. The remaining distal 0.4 to 0.5 cm. of muscle tissue was whiter, with a roughened surface and a necrotic appearance. The tip was roughened fragments of tissue averaging 0.7 mm. in diameter, from which projected numerous fine, white strands like fibrous tissue. Instead of the healing process evidenced



A and *B* indicate mural fragments of the ruptured posterior mitral papillary muscle; *C*, necrotic fragment of the papillary muscle attached to the valve leaflet; *D*, ruptured chordae tendinae.

in the other fragment, there appeared to be a necrosis with shrinkage of the tissue of this fragment.

On the right of the conical stump of the papillary muscle and 0.6 cm. above it, was another papillary muscle stump 0.5 cm. long and averaging 0.4 cm. in diameter (*B* in illustration). The apex of this was similar to the one first described, was conical, covered by gray, shiny tissue-like endocardium, and the tip was turned in forming a ring 0.3 cm. in diameter. On the same posterior leaflet of the mitral valve, just to the left, was a chorda tendina, 1.3 cm. long and 0.5 mm. in diameter, which appeared to have been torn from this fragment of papillary muscle (*D* in illustration). The distal tip of this

chorda tendina was necrotic, the surface irregularly roughened. There were four shorter fragments of chordae tendinae which seemed to have been torn from the former and averaged from 0.3 to 0.5 cm. in length. The circumference of the tricuspid ring was 12 cm. There was a mural thrombus of the right auricula 4 by 3 by 2 cm. The aortic ring was 6 cm. in circumference. There was a linear wrinkling of the root of the aorta and a slight aneurysmal bulging, the wall of which was thin. In 13 cm. of the thoracic aorta there was a similar linear wrinkling. There was a marked generalized arteriosclerosis. In sections, there was a moderate fibrous myocarditis, and in those of the aorta the intimal side of the media was irregularly roughened and necrotic; the space between was filled with disintegrating cells and blood. Around many of the blood vessels of the adventitia and media, there was a small round cell infiltration, changes like those due to syphilis.

Anatomic Diagnosis: Healed rupture of the posterior papillary muscle of the mitral valve; marked hypertrophy of the myocardium of the left ventricle of the heart; fibrous changes of the anterior mitral leaflet; mural thrombosis of the right auricula; recent embolus of the right branch of the pulmonary artery; acute emphysema of the lungs; syphilitic sclerosis and fatty changes of the aorta; chronic diffuse nephritis (arteriosclerotic kidneys); chronic passive hyperemia of the viscera; fibrous myocarditis; etc.

In all of the reports mentioned except the one by Heiman, the papillary muscles torn are of the mitral valve. In four reports (Bertin, Dennig, Wankel, and Teacher) there was a marked sclerosis of the coronary arteries, and in two (Wankel and Dennig) a thrombosis of the coronary arteries. Courvisart makes no mention of either condition, and in Legendre's report the coronary arteries were normal. In one (Bertin) the chordae tendinae were covered with vegetations. Syphilis seems to have been the cause in the report by Spaulding. In no instance has death occurred immediately at the time of rupture but hours or days later, and usually because of some secondary factor.

There seems to be no other report of complete healing of a torn papillary muscle. This is a distinctive feature of the heart described here. Careful examination of the coronary arteries in this heart demonstrated a marked sclerosis but no thrombosis. The etiology of the rupture is therefore obscure but it may be syphilitic.

Spontaneous rupture of the papillary muscles of the heart is rare. When it occurs the lesion usually is of the mitral valve. Death usually follows some time after the accident. There is no one etiologic factor, nor are there definite symptoms.

MALIGNANT TUMOR OF THE THYMUS WITH EXTENSIVE METASTASES. DR. SETH E. BROWN.

A man, aged 36, was admitted to the Research Hospital of the University of Illinois with the diagnosis of carcinoma of the thyroid gland and mediastinal tumor. The patient's condition became progressively worse, and he died five months after the onset of the first symptoms.

Postmortem examination by Dr. R. H. Jaffé demonstrated a large tumor mass in the anterior superior mediastinum with metastases to the lungs, heart, thyroid gland, cervical glands, liver, pancreas, kidneys, suprarenals and mesenteric lymph glands.

Microscopically, the primary growth contained much hyalinized fibrous tissue with few tumor cells, while the metastases were cellular. The cells

varied greatly in size and shape, the nuclei were large and vesicular, and fine protoplasmic branches extended from cell to cell. In places the cells were arranged about minute alveoli. No Hassall's corpuscles were found. The tumor is diagnosed as reticulum cell lymphosarcoma.

DISCUSSION

R. H. JAFFÉ: The tumor demonstrated by Dr. Brown shows how difficult it sometimes may be to determine whether a tumor is sarcoma or carcinoma. All the histologic criteria characteristic of sarcoma or carcinoma are of no value with a tumor composed of immature cells. In the metastases to the lymph vessels of the lung, to the alveoli of the lung and to the vesicles of the thyroid, tumor cell borders on tumor cell, and there is no fibrillar reticulum demonstrable. In the places, however, where the cells break into the stroma, they mingle so intimately with the latter that most of the cells are embedded in a network of fibrils. Occasionally, especially in the pancreas, there are small alveoli without intercellular fibrils (Bielschowski's stain). When we call the tumor a reticulum cell lymphosarcoma we do so on account of the branched shape of the cells forming a protoplasmatic reticulum besides the fibrillar one. This protoplasmatic reticulum also is present in the alveoli. In the periphery of the alveoli the protoplasmatic branches joint the fibrillar structures.

The term thymoma should be restricted to the tumors of the thymic region of undoubtedly epithelial origin containing Hassall's corpuscles.

SKIN REACTIONS WITH GONOCOCCUS TOXIN. RUSSELL D. HERROLD. (John McCormick Institute for Infectious Diseases.)

Positive skin reactions with gonococcus culture filtrates were reported with dilution 1:100 and less. Recently, reactions have been noted with various filtrates in dilutions 1:500 and 1:1,000. Ordinary broth is the medium with the exception of the addition of 0.1 per cent dextrose and the substitution of 0.1 per cent dibasic sodium phosphate for the sodium chloride.

The cultures were made in large flat bottles containing broth to a depth of about 1 cm. The bacteria grew abundantly and at the end of seven days the cultures were filtered through Berkefeld N filters. Subcultures of the seven day growth demonstrated that the gonococci were viable.

Gonococci grown on solid medium and then added to broth were set aside for seven days in order to autolyze. This control broth was filtered and skin tests made. No reactions were found in any instance with a dilution of 1:500, and but few with a dilution of 1:100. A comparison by stains of the amount of autolysis of the two types of gonococcus antigen indicated that the amount of autolysis was about the same. These results seem to indicate that there is a toxin produced by the growth of the gonococcus in this medium which differs from that due to autolysis.

Skin tests have been made in children and adults with gonorrhea. Skin reactions are negative or reduced in intensity after a series of subcutaneous injections of the gonococcus toxin. Frequently there is a reduction in the intensity of reaction after a cure. Patients with persistent strongly positive reactions after the clinical symptoms have subsided seem more likely to have later exacerbations of gonorrhea, and thus the test may be of value in the determination of latent infections. The serums of patients receiving a series of subcutaneous injections of the toxin contain some antitoxic substance, as indicated by the neutralization tests. The injection of the toxin subcutaneously seems to decrease the susceptibility toward infection. However, the number of observations is too limited for final conclusions.

Book Reviews

BUCHANAN'S TEXT-BOOK OF FORENSIC MEDICINE AND TOXICOLOGY. Ninth Edition. Revised and Enlarged by JOHN E. W. MACFALL, M.D., D.P.H. (LIVERPOOL); M.B.; CH.B. (VIET.); PH.C., Professor in Forensic Medicine and Toxicology, University of Liverpool; Honorary Adviser to the Criminal Investigation Departments, Liverpool and Birkenhead Police, etc. Cloth. Price, $5. Pp. 445, with 57 figures. New York: William Wood & Company, 1925.

The favor which this one of a number of shorter treatises on forensic medicine by English authors has enjoyed is evident from the number of the editions. In this edition the recent statutes of Great Britain concerning the sale, labeling, prescribing, etc., of poisons, a brief chapter on "Professional Conduct" and an account of the legal restrictions for cremation have been added. The many tables, particularly those in the section on toxicology, and the illustrations of crystals by which many poisons are identified, are all excellent.

Greater care should have been employed in reading proof. Some topics discussed have no place in the index at the end of the book. There are no references in the text to many illustrations; references to other illustrations are many pages distant. A little speculation may be excusable over what happened when after being shot a woman prowled about the house and dropped "the bullet from her combinations on the stairs" (p. 199). In the paragraph devoted to phosphoretted hydrogen (p. 431) this appears: "It may produce rapid, followed by slow and labored breathing and convulsions." Measurements are in lines and inches, and some poisonings, such as those from male fern, strophanthus, amyl nitrite and emetin are not considered. There is abundant opportunity in subsequent editions in discussions of a number of subjects, for more notice of modern investigations in well organized institutes of legal medicine in other parts of Europe, South America, and elsewhere.

In spite of such defects, the work is a worthy one, especially for students of medicine and law in Great Britain. Its compactness alone is a great merit. The instructions for postmortem examinations issued by the Crown Office in Scotland to Medical Inspectors are interesting reading in view of the general absence in the United States of anything approaching official regulation of such details.

CLINICAL INTERPRETATION OF THE WASSERMANN REACTION. By ROBERT A. KILDUFFE, A.B., A.M., M.D., Director of Laboratories, Atlantic City Hospital. Philadelphia: Lea and Febiger, 1926.

Dr. Kilduffe has assembled many facts which are important in interpreting the results of the Wassermann test for application in the diagnosis of syphilis for the individual patient. As the author states, the ground covered is familiar to the serologist and to the trained syphilologist, but is less well understood by physicians in more general lines. The subject matter is well organized and presented in an interesting, logical and easily readable manner. The principles and mechanism of the test are briefly though sufficiently discussed, and the relation of the test to the stages of syphilitic infection is elucidated so far as it is known. The occurrences of false positives and of false negatives are taken up, as are also numerous conditions which have occasionally been

reported as factors in producing false results, such as pregnancy, tuberculosis, diabetes, etc. There are also chapters on the importance of provocative tests and on reactions in neurosyphilis and congenital syphilis. In the latter, the reliability of reactions on placental and cord bloods is supported. The author recommends the use of more than one antigen, and emphasizes the importance of quantitative methods as general safeguards. In its latter half, the book becomes a eulogy of the Kolmer test. The reader is told that this test is the most delicate and specific at present available. In fact, it is stated that aside from yaws, false positive reactions do not occur with the method, and much carefully selected data are reviewed and tabulated to support the claim. It is asserted that false positives obtained by other systems are self indictments of their reliability as methods. The final chapter deals with the proper collection of test samples for the serologist.

A Manual of Clinical Laboratory Methods. By Clyde Loltridge Cummer, M.D. Second edition. Philadelphia: Lea & Febiger, 1926.

Here is much carefully selected information concerning clinical laboratory methods. The illustrations are good; the book is remarkably free from typographic errors, although there a few gross errors in labeling the illustrations; and the tabulations are not all explicit.

The plates illustrating types of blood cells are excellent. In addition to the subjects usually considered in such manuals, there are chapters on immunologic, bacteriologic and chemical methods which are complete and excellently written. A feature of especial value is the frequent reference to original articles and monographs.

While the specialist in any particular branch of the subjects considered would wish to add paragraphs here and there, for general use the book is sufficiently comprehensive. Indeed, the amount of exact information recorded in such a small volume is amazing, and as one browses through the pages enthusiasm for it grows.

The two chapters on blood are not quite up to the standard set by the authors, and might well be enlarged. One might well take issue with the unmodified statement that "all cells of myeloid origin . . . . show blue granules" with the oxidase stain. The only oxidase method included is rather complicated and without reference to several simpler methods.

The book is recommended for those who have already had detailed instruction in clinical laboratory methods and especially as a vademecum for the intern entering the laboratory service.

The Biology of the Protozoa. By Gary N. Calkins, Professor of Protozoology, Columbia University. Cloth. Price $7.50. Pp. 623. Philadelphia: Lea & Febiger, 1926.

Professor Calkins feels that our knowledge of the protozoa has been gained from a vast accumulation of facts from many different aspects (such as taxonomy, morphology, pathology, physiology, etc.) without any common point of view other than that they are organisms of one cell. Even this concept, which has been questioned by some, he feels has not been a "unifying conception." He, therefore, goes deeper and selects as his underlying biologic principle "the irritability of protoplasm, combined with protoplasmic organization" and attempts by his presentation to furnish a groundwork for a science of protozoology. It is impossible, in this brief space, to trace even the general outlines of Professor Calkin's concepts, but it is evident that he is attempting

to review and correllate the many facts of protozoology from a physiologic and general biologic standpoint. This is indeed a healthy point of view when one considers the many "special systems" that have arisen in protozoology during the last two decades which seemingly have little in common with well established concepts of general biology. It is somewhat disappointing, however, that so little attention is paid to the biology of parasitism and the parasitic protozoa.

The subject matter is indicated by the chapter headings. Following an introductory account of the distribution and general organization of the protozoa, there are four chapters dealing with nuclear and kinetic elements, structural differentiations, general physiology and reproduction. Then there are four chapters dealing with the special morphology and taxonomy of the four groups of the protozoa, followed by three chapters on vitality, phenomena accompanying fertilization and the effects of reorganization and the origin of variations in the protozoa. The last three chapters are the ones that will be of particular interest to protozoologists, because they represent the author's chief field of research during recent years and give in detail his conclusions in regard to the effects of asexual reproduction, endomixis and conjugation.

The book is beautifully illustrated, largely with figures from the author's publications. These would, however, be of more value had their magnifications been indicated. A very useful bibliography is given at the end of the book, as well as a short selected bibliography at the end of each chapter.